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THE QUANTITATIVE DETERMINATION OF THE SERUM BILIRUBIN WITH SPECIAL REFERENCE TO THE PROMPT-REACTING AND THE CHLOROFORM-SOLUBLE TYPES

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SINCE the original description by van den Bergh and Snapper[18] of a method for the quantitative estimation of bilirubin, based on the Ehrlich-Proscher diazo reaction, many modifications have been advocated.[2-10, 32] All of these have represented an attempt to avoid the three main sources of error inherent in the original method: namely, (1) loss of bilirubin by adsorption on the protein precipitate; (2) variations in the azobilirubin color due to changes in pH; and (3) imperfect color match with the standard color solution originally recommended.

Regarding the qualitative aspects of the color reaction, van den Bergh and Müller,[11] in 1916, first noticed the differing behavior of the "direct"- and "indirect"-reacting serum bilirubins, which has subsequently become so well known. In 1934 van den Bergh and Grotepass[7] described a colorimetric method for the quantitative evaluation of direct and indirect fractions of the serum bilirubin. This publication likewise, was followed by a number of proposed modifications.[9, 12-14]

Of all of the methods which have been proposed, the one described by Malloy and Evelyn[9] has met the most general acceptance, since it permits an accurate estimation of total bilirubin and a ready distinction between the direct- and indirect-reacting fractions. Unfortunately, Malloy and Evelyn recommend reading the total direct-reacting bilirubin only after 30 minutes as a routine procedure, and for a more detailed study of the behavior of the direct reaction they recommend readings at 10, 30, 60, and 120 minutes. As will be emphasized in detail later, the 10-minute reading already combines the prompt and delayed types, which have entirely different significance, since the available evidence indicates that the delayed direct- and the indirect-reacting types are much more nearly related and that the delayed type should, accordingly, be estimated separately. Even the 5-minute reading as proposed by Cantarow and his associates[15] has the same disadvantage.

The time limit for the prompt as compared with the delayed is bound to be somewhat arbitrary. Based upon studies of bile bilirubin and sera from cases of mechanical jaundice, on the one hand, as contrasted with hemolytic

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jaundice sera or hemorrhagic bilirubin containing fluids from body cavities on the other,[1b] it appears certain that nearly all of the prompt-reacting type give rise to azobilirubin formation within 1 minute, while the delayed direct-reacting type has not yet begun to produce color at this time. Admitting that there may be slight overlapping in some instances, it is nevertheless certain that a division at 1 minute offers the greatest likelihood of a sharp separation of the two forms when the basis of separation is speed of reaction only.

The separate measurement of the direct- and indirect-reacting bilirubins has been approached from a different angle following the first report and subsequent papers of Grunenberg[16-18] who noticed that the indirect-reacting bilirubin is soluble in chloroform, while the direct-reacting type is not. His observations have been confirmed by a number of investigators, and some of them have used this differing behavior for the quantitative estimation of both types of bilirubin.[19-21] Heilbrun and Hubbard[22] have applied the chloroform solubility of the indirect-reacting bilirubin to perform an improved icteric index. No mention has been made of the question of chloroform solubility of the delayed direct-reacting bilirubin.

The description by Feigl and Querner[23] of the so-called biphasic reaction complicated the interpretation of the qualitative van den Bergh. McNee[24] believes that this type of reaction is due to the presence in the serum of certain proportions of both direct- and indirect-reacting bilirubins. We believe the biphasic reaction is better explained by the relative amount present of prompt-reacting and delayed direct-reacting bilirubins. As noted, however, the latter is believed to have the same significance as the indirect-reacting bilirubin. Hence, it is important to differentiate both types of direct-reacting bilirubins in any quantitative method. This is easily achieved by a slight modification of the Malloy and Evelyn technique consisting simply of making the reading for the prompt direct-reacting bilirubin at 1 minute after the addition of the reagent to the diluted serum.[25] The total direct-reacting bilirubin is read at 15 minutes. In some sera with a high content of the delayed direct-reacting bilirubin some of it is unable to react within 15 minutes, but this is of little moment since any that does not react directly will be picked up in the total reaction following addition of alcohol. The 15-minute period is purely arbitrary, since the longer one waits, the more of the so-called indirect-reacting bilirubin will react directly, that is, without addition of alcohol. Actually it may be superfluous to measure the delayed or 15-minute value at all, and we believe that this can be omitted without seriously affecting the information derived, either from a clinical or fundamental standpoint. We have, however, retained the 15-minute delayed reading for purposes of comparison, as given below, and we feel that it has some value in serving as a dividing zone between the pure prompt type reacting within 1 minute and the pure indirect which has not reacted after 15 minutes except with the aid of alcohol. In other words, the 15-minute value permits a sharper separation of the prompt-reacting and the indirect-reacting types. The reading for the total bilirubin is made 15 minutes after the addition of alcohol.*

To recapitulate:

1. The 1-minute reading: prompt direct-reacting bilirubin.

*Malloy and Evelyn allowed a 30-minute period after addition of alcohol. Our experience has shown that the values are the same after 15 minutes as after 30 minutes.

2. The 15-minute reading: total direct-reacting bilirubin.
3. The difference between the 1- and 15-minute reading: delayed direct-reacting bilirubin.
4. The 15-minute reading after adding alcohol: total bilirubin.
5. The difference between this and the reading for total direct-reacting bilirubin: indirect bilirubin.

Cantarow and co-workers,[26] on the basis of their study of the direct (30-minute)-reacting bilirubin, comment: "It is obvious that the production of the qualitative direct reaction, at least in serums of relatively low bilirubin content, is not determined by the concentration of direct-reacting bilirubin or by the proportion which it constitutes of the total. It must be remembered, however, that readings of the qualitative reactions are made at 30 minutes, which may be responsible, in part at least, for the discrepancy." These investigators found higher values for direct-reacting bilirubin (30 minutes) in sera from patients with cirrhosis or mild degrees of hepatitis than in the controls, both groups having a normal concentration of total serum bilirubin. These results have not been confirmed by others.[27] Measuring the prompt (1-minute) bilirubin, Delgado[28] found that the type of qualitative van den Bergh reaction depends upon the amount of prompt bilirubin present as can be seen in Table I.

TABLE I

CORRELATION BETWEEN THE AMOUNT OF DIRECT-REACTING BILIRUBIN AND THE TYPES OF THE QUALITATIVE VAN DEN BERGH REACTION (DELGADO[28])

NUMBER OF DETERMINATIONS	QUALITATIVE VAN DEN BERGH	PROMPT BILIRUBIN (1 MINUTE) RANGE (MG. PER 100 C.C.)	TOTAL BILIRUBIN RANGE (MG. PER 100 C.C.)
50	Indirect	0.00 to 0.15	0.70 to 16.04
16	Delayed biphasic	0.15 to 0.88	0.63 to 3.27
40	Prompt biphasic	1.01 to 6.98	2.60 to 17.18

Sepúlveda and Osterberg[29] have recently applied the method of Varela-Fuentes and Recarte[20] to the photelometer (Sheard-Sanford) in order to measure the chloroform-soluble bilirubin. The values for direct-reacting bilirubin are obtained in dilutions according to the Malloy-Evelyn method, but this is determined only when the qualitative van den Bergh reaction is prompt or biphasic, and even then the time of the determination is rather indefinite, being "made during the first 10 minutes after alcohol is added."[29] The evident purpose of this is to avoid the error due to slow coupling of the indirect-reacting bilirubin with the diazo reagent even in aqueous solution. It has appeared to us that the use of the 1-minute reading with the Malloy-Evelyn method, as already referred to, makes procedures of the Sepúlveda-Osterberg type quite superfluous, since the former is much simpler and is not liable to variables which may not be recognized, such as loss incident to chloroform extraction, emulsion, heating, and other factors.

The purpose of the present investigation, therefore, has been a comparison of the described modification of the Malloy-Evelyn method with the Sepúlveda-Osterberg procedure. An additional purpose was to study the criticisms of the Malloy-Evelyn method recently advanced by With.[30]

EXPERIMENTAL

The Malloy-Evelyn method, with the time modifications noted in the foregoing, was compared with the Sepúlveda-Osterberg method on forty-one human blood sera of varying bilirubin content. All readings were made with an Evelyn photoelectric colorimeter, calibrated in the usual manner with crystalline bilirubin (see Malloy and Evelyn[9]). The center setting for the Sepúlveda-Osterberg modification was obtained with the same mixture of chloroform-alcohol which they recommend for redissolving the dry residue after the chloroform extraction rather than with water as suggested by them. As will be seen in Table II, the values for the chloroform-soluble bilirubin obtained with their method were, for the most part, lower than those obtained by calculating the difference between the total bilirubin and the total direct (15-minute) value and were, of course, much lower than the difference between prompt and total.

TABLE II

COMPARISON BETWEEN SEPULVEDA-OSTERBERG AND MALLOY-EVELYN PROCEDURES
(VALUES IN MG. PER 100 C.C. OF SERUM)

SERUM	SEPULVEDA-OSTERBERG ($CHCl_3$ SOL.)	1 MIN.	PER CENT OF TOTAL	15 MIN.	PER CENT OF TOTAL	TOTAL	INDIRECT
1	0.33	0.74	34.2	1.48	68.5	2.16	0.68
2	0.44	2.96	72.0	3.50	85.3	4.10	0.60
3	1.00	5.20	63.8	6.80	83.5	8.14	1.34
4	1.30	4.28	57.3	6.12	82.0	7.46	1.34
5	0.99	0.23	15.3	0.62	41.3	1.50	0.88
6	1.01	8.28	46.8	10.76	60.8	17.68	6.92
7	0.72	0.62	40.0	1.23	79.3	1.55	0.66
8	0.49	3.94	50.0	5.58	70.8	7.88	2.30
9	2.40	9.90	52.8	13.40	71.5	18.74	5.34
10	0.09	0.12	19.0	0.32	50.8	0.63	0.31
11	9.90	0.76	5.0	1.60	12.1	13.20	11.10
12	0.20	0.86	35.8	1.60	66.6	2.40	0.80
13	1.70	4.38	47.2	6.60	71.1	9.28	2.68
14	0.40	0.67	54.0	0.96	77.4	1.24	0.28
15	0.87	2.00	50.5	2.96	74.7	3.96	1.00
16	0.86	2.28	31.2	4.60	63.0	7.30	2.70
17	2.90	6.54	28.0	13.20	57.9	22.80	9.60
18	1.80	0.50	13.8	0.90	25.0	3.60	2.70
19	2.10	0.57	19.7	1.37	47.5	2.88	1.51
20	0.88	4.46	69.6	4.82	75.3	6.40	1.58
21	1.10	4.16	57.6	5.58	77.2	7.22	1.64
22	0.76	3.44	57.3	4.68	78.0	6.00	1.32
23	1.09	5.39	60.3	7.08	79.1	8.94	1.86
24	0.40	1.47	40.8	1.87	51.9	3.60	1.73
25	0.95	0.28	16.1	0.67	38.7	1.73	1.06
26	0.51	3.82	65.8	4.60	79.3	5.80	1.20
27	0.47	0.38	33.3	0.81	71.0	1.14	0.33
28	0.40	5.20	57.7	7.46	82.8	9.00	1.54
29	2.51	13.94	56.2	17.28	69.6	24.80	7.52
30	0.07	0.55	50.0	0.77	70.0	1.10	0.33
31	1.31	5.44	46.8	7.53	64.8	11.60	4.08
32	0.24	0.11	22.0	0.22	44.0	0.50	0.28
33	0.72	1.27	55.2	1.32	57.3	2.30	0.98
34	0.27	4.02	46.9	6.38	74.5	8.56	2.18
35	0.83	2.46	47.3	3.78	72.6	5.20	1.42
36	0.36	1.20	50.0	1.86	77.5	2.40	0.54
37	1.33	11.36	61.0	14.72	79.1	18.60	3.78
38	3.00	0.40	8.8	0.90	20.0	4.50	3.60
39	0.20	0.24	31.6	0.38	50.0	0.76	0.38
40	0.72	2.01	47.8	2.76	65.7	4.20	1.44
41	4.20	0.83	11.2	1.24	16.8	7.38	6.14

The question arose as to the efficiency of extraction of the indirect-reacting bilirubin by the chloroform. This was studied in the following way: 1 c.c. portions of various sera were extracted exhaustively with chloroform; this could be ascertained because the chloroform became colorless and the dry residue of the last extract when redissolved in chloroform and alcohol gave a negative van den Bergh reaction. The supernatant fluid after the chloroform extraction was then treated according to the modified Malloy-Evelyn technique and, as noted in Table III, an important fraction of the indirect-reacting bilirubin was still present; in other words, the chloroform extraction was not particularly efficient.

TABLE III

VALUES FOR PROMPT AND DELAYED DIRECT-REACTING AND TOTAL BILIRUBINS IN THE SUPERNATANT FLUID OF SERA EXTRACTED EXHAUSTIVELY WITH CHLOROFORM

SERUM	1-MIN. VALUE IN MG. PER 100 C.C.	15-MIN. VALUE IN MG. PER 100 C.C.	TOTAL IN MG. PER 100 C.C.
a	2.12	3.08	4.24
b	0.17	0.32	0.42
c	1.08	2.01	3.00
d	1.65	1.87	2.43
e	0.99	1.27	2.03
f	0.37	0.75	1.5
g	0.18	0.47	0.85
h	2.59	4.32	4.02
i	4.62	7.73	9.25
j	0.52	0.91	3.6

With[30] has recently stated that the Malloy-Evelyn method gives low values because the excessive dilution slows the reaction. He compared this method with Jendrassik and Grof's procedure[10] but added alkali (Fehling II) in both instances; he concluded that results obtained with the Malloy-Evelyn method are from 25 to 50 per cent lower. This was explained as being due to a weaker catalytic activity on the part of methyl alcohol than of the caffeine-sodium benzoate used by Jendrassik and Grof.

In order to study this point, the values for total bilirubin were obtained in four sera using the Malloy-Evelyn procedure and also using caffeine-sodium benzoate instead of alcohol. Readings were first made without addition of alkali, after which 1 c.c. of the Fehling II solution was added to each tube and readings were again taken, this time with a 520 filter rather than the 540 filter as regularly used. This change is also in accordance with the Jendrassik-Grof method. The data are given in Table IV where it is seen that the values obtained when methyl alcohol was used are uniformly higher than those when caffeine-

TABLE IV

VALUES FOR TOTAL BILIRUBIN USING METHYL ALCOHOL AND CAFFEINE-SODIUM BENZOATE WITH AND WITHOUT ADDITION OF ALKALI

SERUM	BILIRUBIN IN MG. PER 100 C.C. (WITHOUT ADDING ALKALI)		VALUES IN EVELYN READINGS* (ADDING ALKALI)	
	METHYL ALCOHOL	CAFF.-SOD. BENZ.	METHYL ALCOHOL	CAFF.-SOD. BENZ.
a	2.88	1.31	96	86[2]
b	6.48	2.24	92	83
c	7.30	3.54	92[1]	83[3]
d	6.00	2.30	94[3]	84

*No calibration was made so that the Evelyn readings are given directly. It must be borne in mind that the higher the reading, the lower the concentration.

sodium benzoate was used, but when alkalinized the reverse is noted. From this it is clear that it is not a matter of differing catalytic effect but rather an effect of the alkali and caffeine on the color intensity of the azobilirubin. This undoubtedly explains the lower values obtained by With for the Malloy-Evelyn procedure when alkali is added. It was not recommended by Malloy and Evelyn, nor was it used in the present study.

DISCUSSION

Analysis of the present results shows that the chloroform-soluble bilirubin as determined by the Sepúlveda-Osterberg method is regularly less in amount than the indirect bilirubin fraction determined by the Malloy-Evelyn procedure. This is probably due to incomplete extraction by the chloroform, evidence for which is presented.

Varela-Fuentes and his associates[31] believe that the extraction of the chloroform-soluble bilirubin is incomplete but that no essential difference exists between the chloroform-soluble fraction and the indirect-reacting bilirubin. Lopez-García and Zelasco[21] extract but once with chloroform, employing this extract in a semiquantitative fashion and recognizing that chloroform extraction is incomplete and that the material extracted may not be an entity.

The quantitative difference between the Sepúlveda-Osterberg method and that of Malloy-Evelyn is more striking if it is taken into consideration that the amount of indirect bilirubin (Malloy-Evelyn) has been calculated from the difference between the total direct-reacting and the total bilirubins. As the delayed direct-reacting and indirect-reacting bilirubins are believed to have the same significance, the important difference from a clinical standpoint is that between the prompt direct-reacting bilirubin and the total. This, of course, gives still larger differences, as noted in Table II. According to the present results, the chloroform extraction method is not reliable as a quantitative procedure, and since it is, in addition, much more cumbersome and time-consuming than the Malloy-Evelyn method, it is not recommended.

The accepted end point for the qualitative prompt direct van den Bergh reaction is 1 minute. This is based upon van den Bergh's original observations on various icteric sera, fluids, and bile. If a quantitative expression of this reaction is desired, it is logical to obtain the first reading at the end of 1 minute, that is to say, to read the prompt direct-reacting bilirubin. The delayed direct-reacting fraction is much more arbitrary in that the longer one waits, the more of the indirect-reacting bilirubin will react directly, that is to say, without alcohol or other catalytic agent. This phenomenon has been observed repeatedly, especially with hemolytic jaundice sera. In general, it is probably sufficient to make use of but two readings; the 1-minute, or prompt, and the total, following addition of alcohol. The 15-minute direct reading may be reserved as a means of separating more sharply the prompt and the indirect fractions. It is quite possible that insufficient attention has been given, in clinical diagnosis, to the ratio between prompt and total bilirubins.

The percentage that the prompt bilirubin represents of the total pigment (Table II) is not at all proportional to the percentage which the total direct-reacting bilirubin represents of the total; this explains the lack of relationship

found by some investigators[26] between the qualitative van den Bergh and the percentage of direct bilirubin.

SUMMARY AND CONCLUSIONS

1. A slight modification of the Malloy-Evelyn technique for serum bilirubin is recommended, which facilitates estimation of the prompt- and delayed direct-reacting types as well as the total bilirubin. The values obtained at 1 minute represent a quantitative expression of the qualitative prompt direct van den Bergh reaction.

2. The bilirubin extracted from the serum by chloroform, according to the method of Sepúlveda-Osterberg, is not equivalent either to the indirect-reacting or the delayed direct-reacting bilirubins. In the majority of instances it is less than either, and in all instances much less than the sum of the two. The lower values obtained by chloroform extraction are due to incomplete extraction. Since the delayed direct and the indirect fractions are believed to have identical significance, chloroform extraction is held to be inadequate and superfluous.

3. The objection of With to the Malloy-Evelyn method, based on the assumption that methyl alcohol has a weaker catalytic activity than caffeine-sodium benzoate, has been found to be valid only for the alkaline solution employed by With and not for the solution prepared by the Malloy-Evelyn technique and used in the present study.

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THE PATHOLOGY OF EXPERIMENTAL CHOLINE DEFICIENCY IN DOGS

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STUDIES on the experimental production of acute choline deficiency in young puppies and of liver function in these animals have been reported by McKibbin, Thayer, and Stare.[1] Standard liver function tests were used, and the results of the tests were correlated with the lipid content of the livers. The present study is concerned with the lesions in these dogs and the correlation of morphologic changes with abnormal liver function tests.

METHOD

Litters of weanling puppies were used in these experiments, and the choline deficient diet was started after a preliminary observation period of ten days. The diets and the liver function tests have been described in detail by McKibbin and co-workers.[1] The chief source of protein was peanut meal, which was used because of its low methionine content.

At the end of the experiment, the puppies were killed by intravenous injection of sodium pentobarbital immediately after the last series of liver function tests had been performed.* Tissues were fixed in 10 per cent formalin solution, and the stains used were hematoxylin-eosin, Sudan IV, and Ziehl-Neelsen (for ceroid[2]).

RESULTS

Data concerning the choline supplementation of the diet, the weights of the liver and kidneys, liver and body weight ratio, liver lipid content, and the results of the various liver function tests are given in Table I. At the time of autopsy, all of the animals which had been fed rations deficient in choline were undernourished. The tendency of the blood to clot was not so evident in deficient as in control animals, and marked pallor of the viscera (anemia) was noted in two of the deficient puppies (Dogs 31 and 32). The livers of the deficient animals were pale yellow or pinkish yellow. The thymuses of the animals which had received no supplements of choline or methionine were invariably smaller than those of littermate controls. In many instances there was no recognizable thymic tissue. When such tissue was present, there was no gross alteration other than the small size of the organ. In Dog. 32 there was edema of the stroma of the thymus.

The microscopic examination revealed consistent abnormalities of the livers and thymuses of the dogs which had been fed diets deficient in choline. The livers of those which had received no supplemental choline or methionine con-

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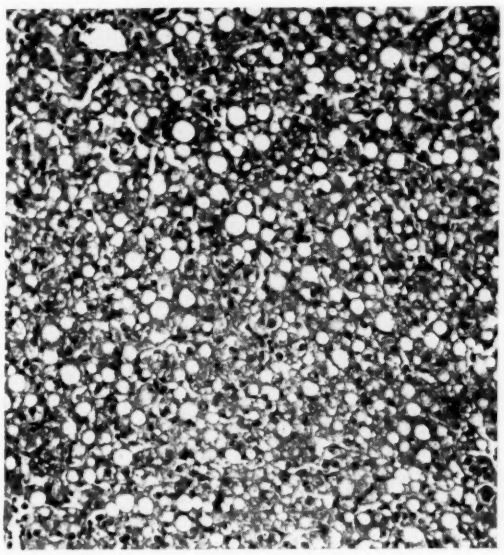
*Dogs 1 to 11 and 24 to 30 are not included in this study because these early diets were deficient in factors other than choline, as indicated by suboptimal growth and abnormalities in the control animals.

TABLE I

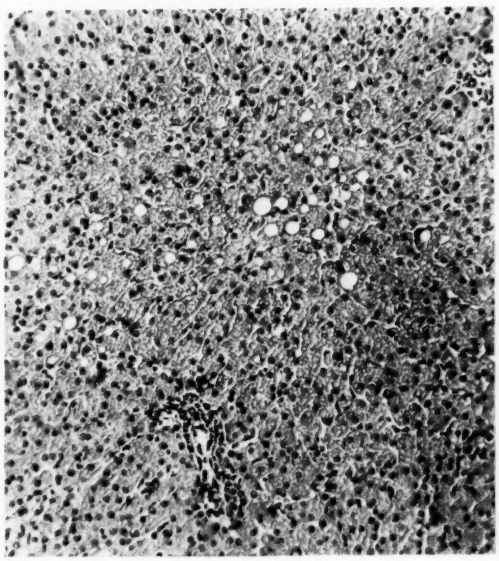
DEFICIENCY OF CHOLINE IN DOGS

LITTER	RATION	DOG	SUPPLEMENT	DURATION OF EXPER. (DAYS)	PER CENT WEIGHT CHANGE DURING EXPER.	LIVER WEIGHT (GM.)	LIVER WEIGHT / BODY WEIGHT × 100	LIVER LIPIDS (CHLOROFORM-EXTRACTABLE) (PER CENT)	KIDNEY WEIGHT (TOTAL)	BROMSULFALEIN TEST (μG. DYE PER ML. PLASMA)	PLASMA PHOSPHATASE (μG. P LIBERATED) 8 HR.	PLASMA PHOSPHATASE (μG. P LIBERATED) 24 HR.	PROTHROMBIN TIME (SEC.)	PLASMA TOTAL CHOLESTEROL (MG./100 C.C.)	PLASMA CHOLESTEROL ESTERS (MG./100 C.C.)
III	3	12	None	43	- 3.0	155	6.1	50.5	28	12	397	998	23.0		
		13	None	43	- 10.4	130	6.0	48.1	22	25	624	1,230	13.0		
		14	150 mg. %	43	+ 49.0	230	7.7	7.4	28	4	122	260	8.0		
		15	150 mg. %	57	+182.0	205	5.5	13.3	27.5	3	79	231	10.8		
		16	150 mg. %	57	+254.0	225	4.2	16.0	43.4	5	87	222	10.8		
IV	3	17	None	32	+ 12.0	120	6.0	47.1	21	31	563	1,090	62.0		
		18	None	32	- 8.5	90	4.3	56.6	18	42	566	1,183	46.0		
		19	None	32	+ 4.9	115	3.8	48.2	25	22	614	1,124	26.0		
		20	0.7% dl-methionine	32	+ 57.0	170	5.0	11.9	29	10	82	245	13.7		
		21	150 mg. %	32	+ 24.6	140	4.9	19.1	24	4	137	349	10.5		
		22	150 mg. %	32	+ 43.2	160	4.5	14.1	27	4	112	300	12.0		
		23	150 mg. %	32	+ 30.1	140	5.9	12.3	23	5	143	399	8.8		
VI	5	31	None	18	- 6.7	129	7.7	34.6	28	25		1,423	55.0	37	9
		32	None	21	+ 9.3	105	7.2	46.3	29	27		1,403	23.8	40	22
		33	10 mg. %	60	+ 71.0	168	4.85	48.7	44	20	629	1,358	16.3	65	37
		34	25 mg. %	60	+ 86.9	184	5.4	43.2	--	20	562	1,098	16.2	39	27
		35	50 mg. %	60	+335.0	235	4.4	25.9	46	9	114	316	11.8	79	58
		36	100 mg. %	60	+441.0	317	4.15	16.9	62	9	72	239	12.9	158	95

tained much lipid material in vacuoles. The vacuoles varied from small to large, the latter being more numerous (Fig. 1, *A*). Supplemental choline chloride in amounts of 10 mg. per 100 Gm. of ration (Dog 33) did not prevent the presence of fat similar in quantity and appearance to that of animals which had received no supplemental choline. One animal which received 25 mg. of choline chloride per 100 Gm. of ration (Dog 34) had appreciably less fat in its liver, and one which received 50 mg. per 100 Gm. of ration (Dog 35) had a liver in which there



A.



B.

Fig. 1.—*A*, Diffuse fatty metamorphosis of liver of Dog 31. (No supplementeal choline.) *B*, Focal fatty metamorphosis in Dog 35 (50 mg. choline per 100 Gm. ration). (Hematoxylin and Eosin, ×150.)

were only small numbers of cells which contained visible fat vacuoles (Fig. 1, *B*). The livers of animals which received suboptimal amounts of choline had relatively few cells which contained large fat vacuoles, but there were minute vacuoles of visible fat in nearly all cells. The large vacuoles were in focal areas in the lobules and were central in some and peripheral in others. One hundred milligrams of choline chloride per 100 Gm. of ration protected the liver against fatty metamorphosis. Equal protection was afforded in the absence of choline by 0.7 Gm. of dl-methionine per 100 Gm. of ration (Dog 20). The livers of several deficient dogs were stained by acid-fast technique for ceroid, and none of this substance was demonstrated.

The thymuses of the puppies fed diets deficient in choline were absent in most instances. Those which were found and examined microscopically differed from the thymuses of the control animals. There were few thymocytes in the medullary portions of the glands of the deficient animals, and the stroma was therefore prominent. The cortices were relatively thin, and the thymocytes were not so densely packed together as in the control animals. The interlobular connective tissue was more prominent in the deficient animals and appeared edematous. The most striking change was in the thymic corpuscles. In the control animals, these were relatively small, and the cells comprising them had homogeneous pink cytoplasm and reticulated nuclei. In the deficient animals, they were represented by large cells often multinucleated. The cytoplasm stained dark pink and vacuoles were frequent. Many had basophilic granular debris within the cytoplasm. The nuclei varied considerably, some being pyknotic and lying in lacunae in the cytoplasm, others being large and pale with aggregations of dark chromatin in focal collections within them. Some had small clear intranuclear vacuoles.

Microscopic examination of brain, heart, lung, spleen, pancreas, adrenal, kidney, testis, ovary, stomach, intestine, eye, and costochondral junction of the choline-deficient puppies revealed no abnormalities.

DISCUSSION

The lesions of the puppies which had been fed choline-deficient rations were not observed in control animals fed similar rations and supplemental choline or methionine. The fact that optimal growth was not obtained from diets which were adequate in all substances but choline is apparent. The cause of the anemia noted in two animals is not known, but presumably it was related to choline deficiency since none of the control dogs was significantly anemic. The anemic dogs were the only ones which had received Ration 5 without supplemental choline.

Although the fatty livers of choline deficiency have heretofore been ascribed to fat infiltration, the irregular distribution of the fat in the livers of the puppies of our series is unlike that of pure fat infiltration in which the fat is characteristically in the periphery of the lobules. In the livers of animals receiving suboptimal amounts of choline, there were invariably some cells which contained large fat vacuoles, but Sudan stains of these livers revealed minute droplets in practically all cells even though fat was not visible in sections stained with hematoxylin and eosin. It seems probable that the lesion of the liver is a com-

bination of both fatty degeneration and fat infiltration. In the livers of animals suffering from marked deficiency, the infiltrated fat may mask that which signifies degeneration.

The diminution of liver function demonstrated by clinical tests (Table I) confirms the anatomic evidence of degeneration. It is unlikely that simple fat infiltration could be responsible for such changes in an organ known to have a factor of safety of approximately 80 per cent. Unpublished experiments from this laboratory on pantothenic acid-deficient puppies reveal little functional abnormality of the liver even though 50 per cent of its dry weight is lipid material. Thus it seems improbable that the mere presence of fat is responsible for diminution of liver function, so that while fatty metamorphosis appears to be an anatomic manifestation of cell injury in choline deficiency, it may only be coincidental.

There were variations in the levels of plasma phosphatase, cholesterol, cholesterol esters, and in bromsulfalein elimination which correlated closely with the morphologic alterations and lipid contents of the livers of the deficient puppies. The prothrombin times of the control animals were uniformly below fifteen seconds, and only one of the deficient dogs had a prothrombin time of less than fifteen seconds. In general, the dogs having large amounts of lipid in their livers had correspondingly high prothrombin times.

The ratio of liver weight to body weight varied considerably in both deficient and control dogs. There was no correlation of this ratio with the presence or absence of choline in the diet. Neither was there correlation between this ratio and the lipid content of the livers. This differs from similar observations on young rats deficient in choline. Studies on these animals have shown a remarkably constant correlation between the relative weights of the livers and the amount of lipid in the livers.[3]

The kidneys of the choline-deficient puppies were normal. This is in marked contrast to the kidneys of choline-deficient rats in which there are profound renal changes. These renal lesions in rats consist of epithelial necrosis of convoluted tubules and Henle's loops, together with marked engorgement of capillaries and subcapsular glomerular hemorrhages.[4, 5] In experiments in this laboratory, these lesions have been produced by a ration similar to the one used on the puppies.[3] Variations of this type are usually ascribed to differences in species, rations, or the ages of the animals used. Since the rations were comparable and the developmental ages of the deficient dogs were presumably the same or less than those of the rats at the beginning of the experiments, we believe this difference to be one of species.

The thymuses of the dogs fed choline-deficient rations were small and there was necrosis of the thymic corpuscles. There were similar changes in the thymuses of the rats which we have studied, and in some there were demonstrable stages in the disintegration of thymocytes. Because of this, the small thymuses are believed to be the result of disappearance of cells together with diminished formation of cells. The latter is indicated by almost complete absence of germinal centers. The mechanism by which deficiency of choline is responsible for these changes is unknown.

CONCLUSIONS

The pathology of uncomplicated choline deficiency in young puppies has been described. There were fatty metamorphosis (degeneration and infiltration) of the liver and atrophic changes of the thymus. The ratio of gross liver weight to body weight did not correlate with the choline content of the diet. The kidneys, and the other tissues examined, of the choline-deficient puppies, were not morphologically abnormal.

The morphologic changes in the liver correlated with impairment in liver function and also support the growth and functional data that the choline requirement of the puppy is between 50 and 100 mg. of choline per 100 Gm. of the ration used.

Control animals fed identical rations supplemented with sufficient choline or methionine were normal in all respects.

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RECOVERY FROM FULMINATING MENINGOCOCCIC INFECTION WITH MYOCARDITIS PROVED BY ELECTROCARDIOGRAPHY

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MENINGOCOCCEMIA with meningitis and associated myocardial and pulmonary involvement is rarely seen. In 1936 Saphir[1] reported two cases of myocarditis with post-mortem findings. In 1939 Hartwell[2] reported another and was able to find a total of twelve such cases in the literature up to that time. All of these terminated fatally. We have been unable to find in the literature any record of recovery from this complication. We were also unable to find a publication which presented electrocardiographic studies in meningococcic myocarditis. The following is a case report and follow-up after six months with electrocardiographic findings of a patient who recovered from meningococcemia with meningeal, myocardial, and pulmonary involvement.

CASE REPORT

A soldier 25 years of age was admitted to an Army hospital in the European Theater of Operations Dec. 24, 1943. He had been well until twelve hours before admission when he developed a chill followed by generalized aches, fever, and drowsiness. Physical examination on admission revealed a well-developed, well-nourished white male who responded clearly to questions. The temperature was 104.2° F. There was no rash on the skin and no petechiae were visible in the conjunctivae. The nasal mucosa and pharynx were mildly reddened. The blood pressure was 120/72; pulse rate, 128 per minute. The pupils were equal and round and reacted promptly to light. Resistance to flexion of the neck was questionable. The Kernig, Brudzinski, and Babinski signs were absent. Deep reflexes were present and equal bilaterally but the abdominal reflexes could not be elicited. On admission, blood was taken for culture which later grew about two hundred colonies of meningococci from 1 c.c. of blood. The white blood count was 27,800 with 92 per cent polymorphonuclear forms. A lumbar puncture revealed clear fluid under normal pressure with no organisms visible on direct smear. This spinal fluid contained nine erythrocytes and thirty-two leucocytes per cubic millimeter, six of the latter being polymorphonuclear forms and twenty-six lymphocytes. The total protein was 24.0 mg. per 100 c.c. and a culture of this fluid was later reported to be sterile. The patient was seen again one and one-half hours later. He complained of intense generalized pain with myalgia most marked above and below the knee joints. There was no redness, swelling, or pain within the joints. No rash was visible on the skin at this time. However, one hour later a number of petechiae were present on the trunk, and nuchal rigidity was now definite. At this time the patient appeared acutely but not critically ill. A diagnosis of meningococcemia with meningitis was made and treatment instituted at once. During the next few hours, the number of petechiae increased until all parts of the body, including the right conjunctiva, were involved. Many of the early lesions soon developed into large purpuric spots. It was evident that we were dealing with a fulminating septicemia—the so-called purpura fulminans.

The initial treatment consisted of 5.0 Gm. of sodium sulfadiazine in 100 c.c. of distilled water injected intravenously. Following this a venoclysis of 2,000 c.c. of glucose-saline solution was started. Intravenous administration of 5.0 Gm. sulfadiazine was repeated in two hours. For a period of five hours there was little change in the soldier's condition except that the blood pressure dropped to 90/60 and although of good quality, the pulse rate was 130. However, four hours later (thirteen and one-half hours after admission) the patient was found

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in a state of circulatory collapse with a fast thready pulse and peripheral cyanosis; the blood pressure was unobtainable. At this time it seemed probable that the patient would die. Since no adrenal cortex extract was available, 5 minims (0.3 c.c.) of 1 to 1,000 solution of adrenalin was injected subcutaneously with a fair response. The venoclysis, which had been discontinued for fear of overburdening the cardiovascular system, was now resumed. Sodium sulfadiazine, 5.0 Gm., was added to the solution and the dose was repeated in two hours. For the next four hours the outcome remained in doubt. The systolic blood pressure ranged between 90 and 94; the diastolic pressure remained at zero for about two hours, then gradually rose to 70. A second injection of 5 minims (0.3 c.c.) of adrenalin was followed by a rise in the blood pressure to 100/70. A few hours later the pressure reached 118/72 and remained normal thereafter.

On the second day of hospitalization the condition of the patient had improved but the peripheral cyanosis persisted. The blood leucocyte count had decreased to 26,300 but the meningeal signs were quite marked. A spinal puncture released cloudy fluid under increased pressure, with 507 erythrocytes and 3,042 leucocytes per cubic millimeter; 76 per cent were polymorphonuclears and 24 per cent lymphocytes. The total protein was 114.4 mg. per cent. The direct smear revealed no organisms and the culture again was sterile. Sulfadiazine by mouth was tried but only the first dose was retained. The first blood sulfadiazine level taken about twenty hours after admission and three hours after the fourth intravenous dose of the drug was 18.6 mg. per 100 c.c. During the second twenty-four hours, ten grams were given intravenously with a rise in the blood level to 21.1 mg. per 100 c.c. free sulfadiazine. On the third day the meningeal signs and myalgia were unchanged. In addition, there was now pain and slight swelling of the right elbow as well as numbness and tingling of both feet. Most of the petechial spots had faded but the large purpuric lesions were still visible. Examination of the chest on this day (December 26) revealed signs of consolidation with bronchial breathing, bronchophony, and increased voice sounds most marked in the right mid-chest posteriorly. Crepitant râles were present over the posterior portion of the entire right side. The temperature was now 100.4° F.; pulse, 124; respirations, 40 per minute. A roentgenogram of the chest taken on a portable x-ray unit revealed extensive areas of increased density throughout both lung fields with only the left base relatively clear. These chest findings persisted for the next two days. On the following day (December 29) an x-ray picture showed marked clearing throughout the right lung and, to a large extent, also the left lung. The lungs were found to be perfectly normal in a roentgenogram taken Jan. 28, 1944. On December 27 several of the largest purpuric skin lesions showed central necrosis. The neck was less rigid with Brudzinski and Kernig signs now absent. Five days later the patient looked and felt quite well. The left foot remained swollen and painful. Weakness of the left anterior tibial muscle was noted. However, following treatment with physiotherapy, apparently normal function of this muscle was soon restored.

ELECTROCARDIOGRAPHIC FINDINGS

The first electrocardiogram was made December 28 (fifth day of hospitalization). This showed a regular sinus rhythm, rate 120, inverted P-waves in Lead I. S_1 amplitude of 1 mm., low voltage of the QRS complex, and upright T waves of low voltage in all leads. There was no elevation of the S-T segments, and the P-R interval was .17 seconds. The second electrocardiogram January 14 showed very distinct changes. There was regular sinus rhythm, rate 100, and P waves in Lead I were now upright and diphasic. The QRS complexes were of greater amplitude in all leads. T_1 was now upright with greater amplitude, S_1 2 mm., T_2 diphasic, T_3 sharply inverted, and S-T segments not elevated in the first three leads. S_4 was absent, T_4 markedly increased in amplitude, and S-T_4 elevated by 2 mm. The third electrocardiogram taken January 20 showed the following differences from the previous one: There was slightly more inversion of T_3 and greater amplitude of T_4. On January 27 the electrocardiogram showed no change from that taken one week earlier. On July 10, 1944, the electrocardio-

graphic studies revealed the following significant changes from those of Jan. 27, 1944: S_1 was now absent, S-T_2 elevated by 1.5 mm., T_2 upright and of normal amplitude, T_3 slightly diphasic but largely inverted. T_4 was now 10 mm. in amplitude, 4 mm. less than previously. Except for the elevation of S-T_2 July 10, the electrocardiographic findings were normal and indicated healing of the previously noted lesion.

COURSE

The temperature on admission was 104.2° F. but following treatment dropped to 99.8° F. within twelve hours. A daily temperature of about 100° F. continued for five days, followed by a normal temperature thereafter. The pulse rate remained above 120 for the first two days with a daily rise above 100 for the first fifteen days of hospitalization. The respiratory rate on admission was 28 but rose to 42 on the third day, returning to normal four days later. The initial white blood count of 27,800 per cubic millimeter dropped to 26,300 within twenty-four hours. The count rose to 42,000 with the appearance of pneumonia on the following day. Thereafter it decreased and became normal on the eleventh day. Except for 2 plus albumin, the first urine specimen was normal. However, during the third and fourth days in the hospital, the urine was grossly bloody with a return to normal on the sixth day. Five days after the second spinal puncture the fluid was clear, under normal pressure, and contained only 6 leucocytes per cubic millimeter. Although arthralgia was present, there was no evidence of effusion at any time. By January 15 the patient wished to be out of bed but because of evidence of myocarditis in the electrocardiogram, bed rest was continued. On January 28 an x-ray of the chest revealed a heart shadow of normal size and on this day the soldier, appearing perfectly well, was transferred to another hospital for further convalescence.

Nearly six months following this transfer the soldier was seen again. He had spent about three months in a hospital convalescing from his illness. Following this he engaged in various types of light duty. At the time of this follow-up examination, however, he had engaged in moderate types of physical labor. He felt well except for slight pain in the muscles of the left leg after moderate exertion. Neurologic examination revealed some loss of epicritic sensation on the dorsum of the left foot and hyperactive deep reflexes. Physical examination was otherwise completely normal. Several of the larger purpuric spots showed superficial scarring. The cardiac response to exercise was excellent —the pulse rate being 76 at rest, increasing to 96 after exercise, and returning to 72 within thirty seconds. The blood pressure was 128/76. An x-ray picture of the chest revealed normal lung fields and a normal cardiac silhouette. The blood count, urinalysis, and sedimentation rate were normal, the last being 2 mm. in sixty minutes.

TREATMENT

During the first twenty-four hours a total of 20 Gm. of sodium sulfadiazine and 5,000 c.c. of glucose-saline solution were injected intravenously. It seemed evident that the danger from the fulminating infection was far greater than any toxic effect that was likely to result from large dosage of the drug. Since the drug could not be retained by mouth, 10 Gm. were given intravenously

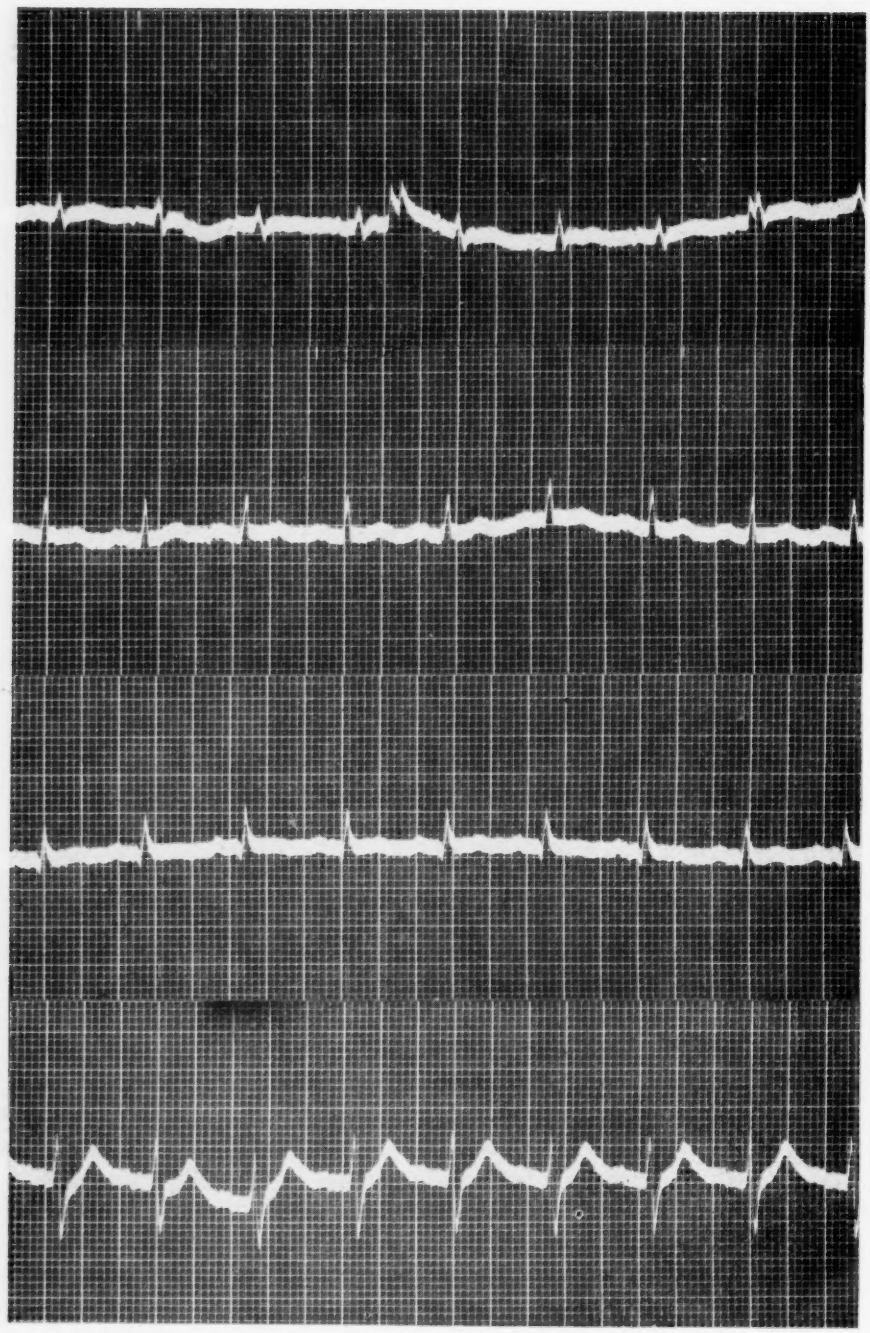

Fig. 1.—Series I taken Dec. 28, 1943.

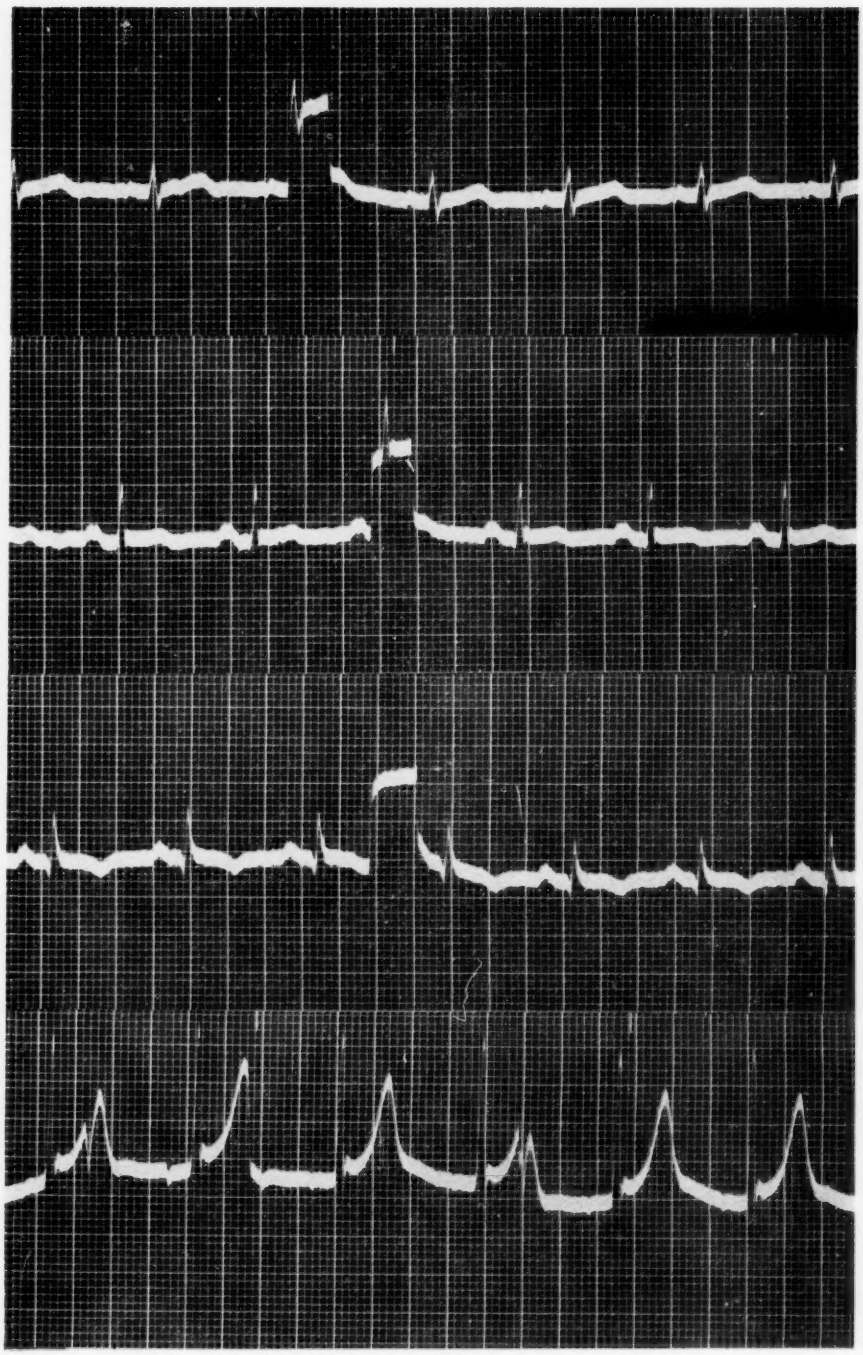

Fig. 2.—Series II taken Jan. 14, 1944.

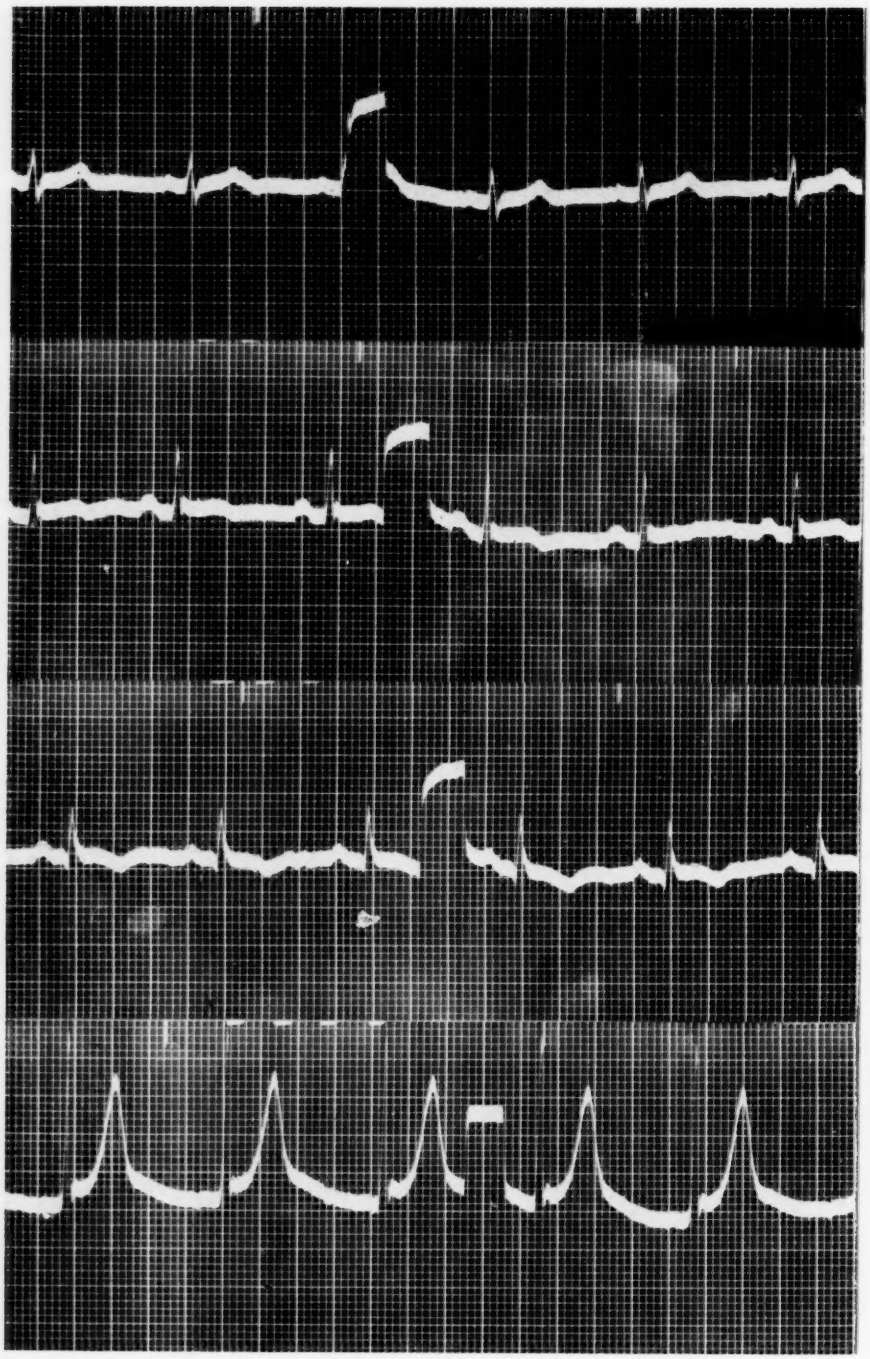

Fig. 3.—Series III taken Jan. 20, 1944.

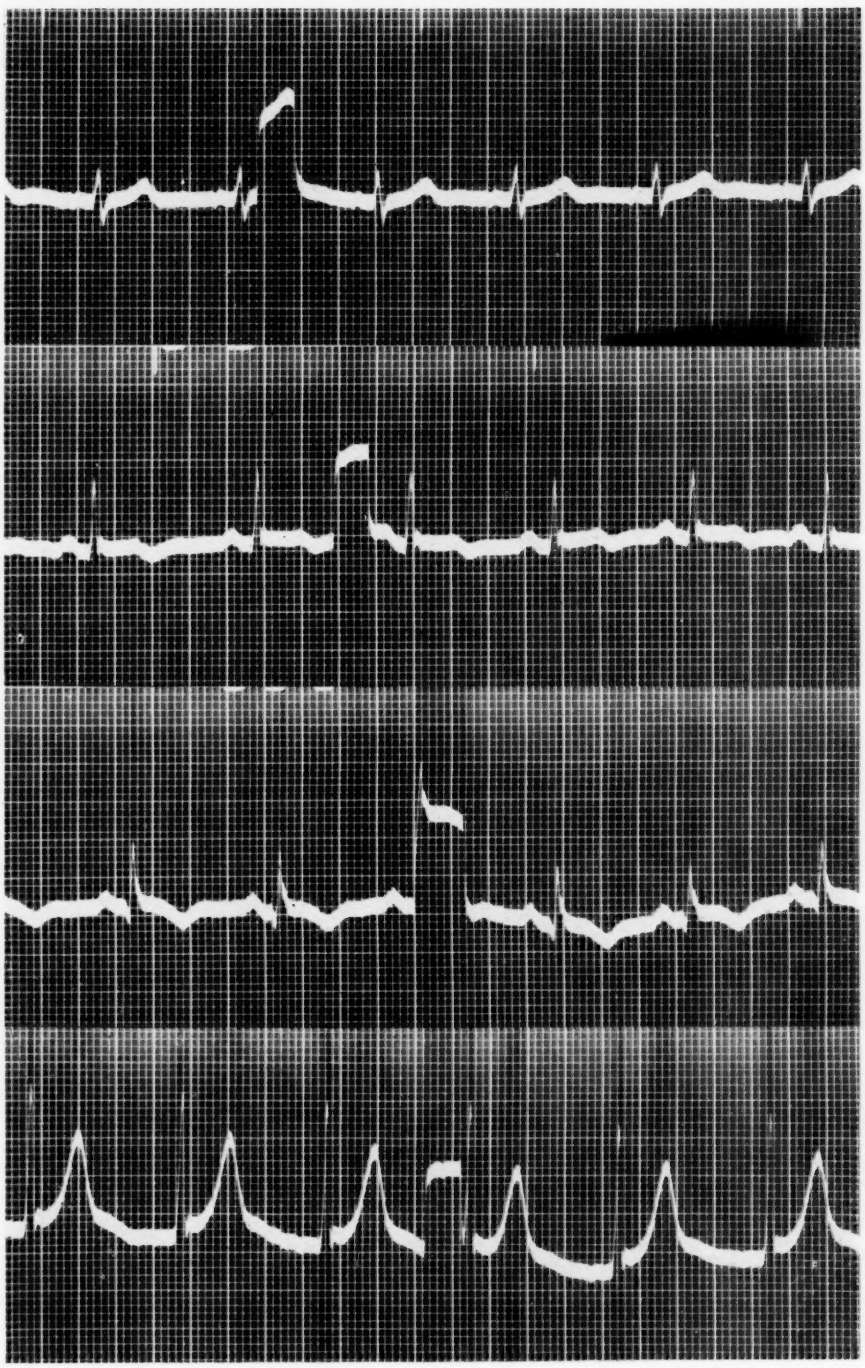

Fig. 4.—Series IV taken Jan. 27, 1944.

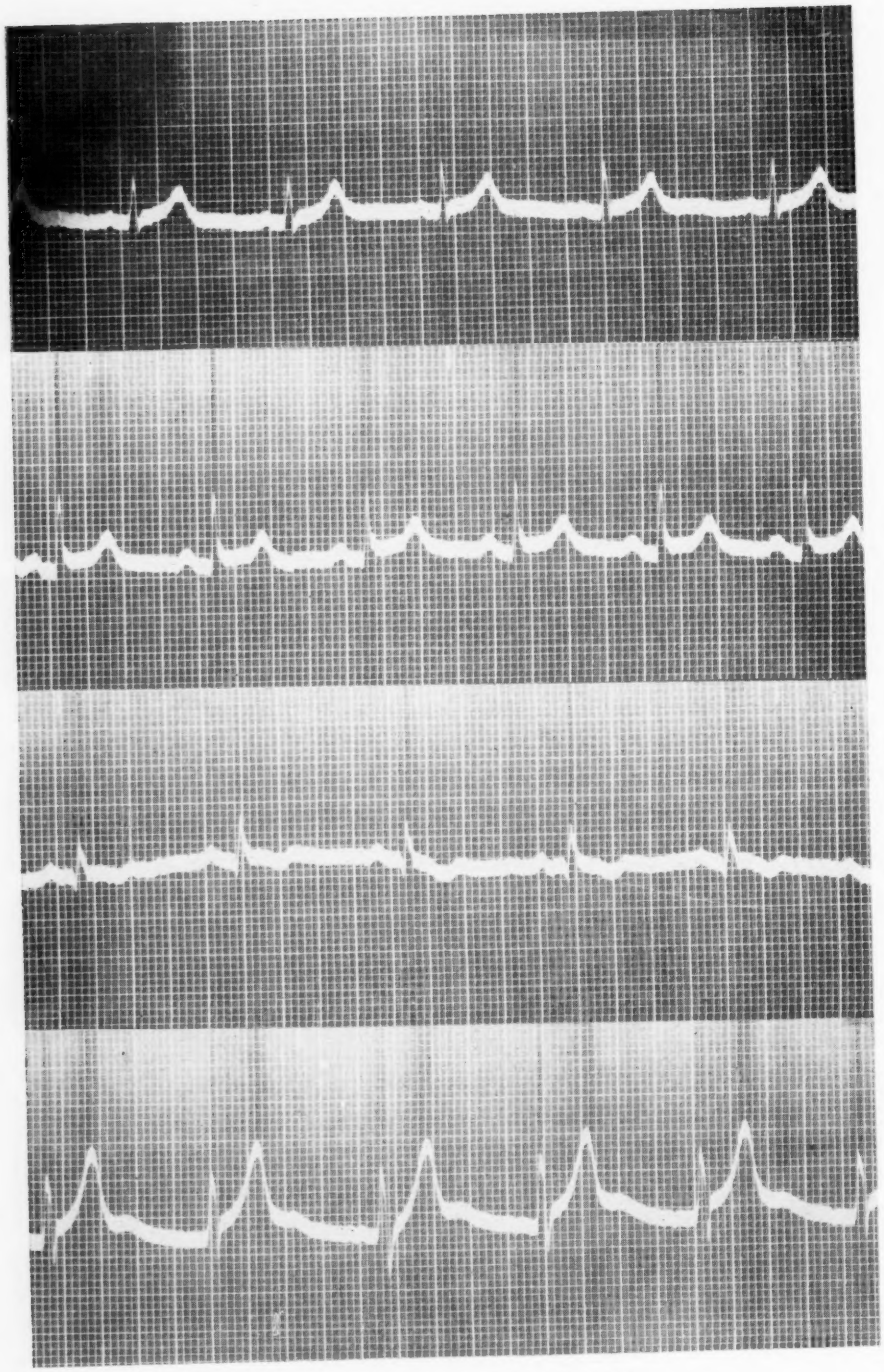

Fig. 5.—Series V taken July 10, 1944.

during the second twenty-four hours in an attempt to maintain the high blood level, but this resulted in a further rise. On the third day with oral medication the level decreased to 14.2 mg. from 21.1 mg. on the previous day. It was maintained at about 6 mg. for the next five days, following which the dosage was reduced again and sulfadiazine therapy discontinued on the twelfth day of hospitalization. There were no apparent ill effects from the drug except for a transient hematuria. Since this cleared in spite of continued sulfadiazine therapy, which was not varied to the slightest degree because of bloody urine, the hematuria may have resulted from hemorrhagic lesions in the kidney due to the meningococcemia itself rather than from a toxic effect of sulfadiazine.

COMMENT

One cannot be sure of the cause of the circulatory collapse which occurred in this patient. Recently Hill and Lever[3] have reported three patients who recovered from this shocklike state following treatment with adrenal cortex extract and intravenous glucose-saline solution. However, these authors made no mention of myocarditis or electrocardiographic studies. We were unable to give adrenal cortex extract because it was not available—yet the patient recovered. Myocardial damage alone could explain the sequence of events, but if the circulatory collapse is explained entirely on this basis, the response to adrenalin and large amounts of intravenous fluid is unusual. The Waterhouse-Friderichsen syndrome with hemorrhage into the adrenal glands probably cannot be diagnosed with certainty except by post-mortem examination. However, the course of the illness during the first sixteen hours closely resembled the clinical course of the other cases in which this diagnosis was later proved at autopsy. The only important variation was the recovery of the patient. Pulmonary involvement is occasionally encountered as a complication of meningococcic infection. The physical findings in our patient were those of multiple small areas of consolidation. Whether or not the consolidation was the result of meningococcic bronchopneumonia, widespread embolization or purpura is a question which must go unanswered.

Information is meager regarding the exact nature of the myocardial involvement associated with meningococcemia. The post-mortem records in the literature indicate that myocarditis alone is rare. More commonly there is an associated endocarditis, and occasionally multiple myocardial abscesses have been found. Our patient presented no clinical evidence of endocarditis. The possibility of myocardial abscesses cannot be excluded. However, with extensive purpura in the skin it is more probable that this hemorrhagic process also involved the lungs and myocardium. The series of electrocardiograms and clinical examination show that the patient had and has recovered from severe myocardial damage.

SUMMARY

1. A case of recovery from a fulminating meningococcic infection with meningeal, myocardial, and pulmonary involvement has been described.

2. Treatment was symptomatic in addition to large doses of sulfadiazine and large amounts of intravenous fluid to combat the toxemia and shock.

3. Adrenal cortex extract was not administered.

4. Follow-up studies after six months revealed electrocardiographic and clinical recovery.

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DIASONE: ITS TOXICITY AND THERAPEUTIC EFFECTIVENESS

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DIASONE, disodium formaldehyde sulfoxylate diamino-diphenyl-sulfone, was synthesized by one of us (G. W. R.) early in 1937.[1] It was prior to this that our attention was attracted by the reportedly high therapeutic effectiveness of 4,4′-diamino-diphenyl-sulfone* in streptococcic infection of mice.[2] But the high toxicity of this drug had been an obstacle in its further chemotherapeutic development. For some years past, we had observed the decisive reduction in toxicity obtained by combining arsphenamine or other arsenicals with sodium formaldehyde sulfoxylate. There was at the same time only a partial reduction in the therapeutic potency, so that the ratio between the maximum tolerated and minimum therapeutic doses shifted in favor of the new combinations.[3] The resulting product, diasone, proved to be considerably less toxic than the parent substance diamino-diphenyl-sulfone. The effect in streptococcic infection in mice remained appreciable.

It is interesting to note that sulfonamides, and particularly diamino-diphenyl-sulfone, have likewise an effect in experimental tuberculosis. Rich and Follis[4] were the first to find that sulfanilamide has an inhibitory influence on infection in experimental tuberculosis in guinea pigs. It was seen by later investigators that diamino-diphenyl-sulfone has an even better effect in experimental tuberculosis. In 1940, Feldman, Hinshaw, and Moses[5] showed also that a derivative, sodium P,P¹-diaminodiphenylsulfone-N,N¹-didextrose sulfonate, designated as promin, had similarly an inhibitive effect on experimental tuberculous infection. In January, 1943, Callomon[6] published a paper in which diasone, when compared to promin and other chemical compounds, was shown to produce the most beneficial results in experimental tuberculosis in guinea pigs. Subsequently Feldman, Hinshaw, and Moses[7] found that diasone is less toxic for guinea pigs than diamino-diphenyl-sulfone and that it is of therapeutic value in experimental tuberculosis, though somewhat less effective than diamino-diphenyl-sulfone. Early in 1943 Petter[8] initiated an extensive clinical trial with diasone which indicated that the drug has a therapeutic effect also in the treatment of human tuberculosis. As a result of his treatment of forty-four patients, he observed that all minimal and moderately advanced cases showed improvement and that there was likewise improvement in a considerable number of far-advanced cases.

From the foregoing it may be noted that diasone has its place in the treatment of experimental and possibly clinical tuberculosis. The purpose of this paper is to establish its pharmacologic properties, particularly its toxicity in lower animals, that is, mice, rabbits, and dogs. Its therapeutic effectiveness in

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*We shall refer to this compound throughout the paper as diamino-diphenyl-sulfone.

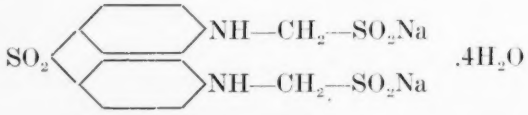
experimental tuberculosis has already been studied by Callomon[6] and also by Feldman, Hinshaw, and Moses.[7] We thought a further extension in the appraisal of its chemotherapeutic properties might be found in the study of the curative effect compared with that of sulfanilamide and sulfadiazine in streptococcic and also pneumococcic infections in mice.

In beta-hemolytic streptococcus infection, Buttle and co-workers[2] found that the single maximum tolerated oral dose of diamino-diphenyl-sulfone for mice is 5 mg. On the other hand, they reported that the rabbit can be given as much as 2 Gm. per kilogram. The effective therapeutic dose of diamino-diphenyl-sulfone was found by the same authors[2] to be 0.4 mg. In 1938 Bauer and Rosenthal[9] determined that the maximum tolerated oral dose of diamino-diphenyl-sulfone for mice is 0.25 Gm. per kilogram, with the therapeutic activity in streptococcic infection of mice thirty times greater than that of sulfanilamide. Feinstone, Bliss, Ott, and Long[10] found diamino-diphenyl-sulfone to be highly toxic for mice and more active than sulfanilamide in streptococcus infection in mice. We have likewise shown that diamino-diphenyl-sulfone was therapeutically active in mice infected with beta-hemolytic streptococcus, but only ten times more so than sulfanilamide.[11]

Diasone is stable and water soluble if protected from oxidation in vacuum glass ampules.[12] Exposed to the air, an aqueous solution after a few minutes turns cloudy. Later a precipitate is formed because of the combined effect of carbon dioxide of the air and the oxidation of diasone, resulting in an increase of the acidity of the solution. By the addition, however, of sodium bicarbonate or sodium hydroxide, the precipitated diasone can be redissolved. If diasone powder is exposed to air for several days, part of it turns insoluble. Mixing diasone and solid sodium bicarbonate (9:1) maintains diasone unchanged and water soluble for a long period of time, provided it is kept in tightly closed bottles.

Gastric juice normally containing from 0.4 to 0.5 per cent hydrochloric acid may act upon diasone, but it was proved by tests that in two hours 0.4 per cent hydrochloric acid at 37° C. causes neither the splitting off of formaldehyde sulfoxylate nor the liberation of diamino-diphenyl-sulfone.

Formed by the reaction between diamino-diphenyl-sulfone and sodium formaldehyde sulfoxylate, diasone cannot be recrystallized and therefore contains some amount of admixture in addition to the major product. The major product represents from 92 to 93 per cent of the substance and corresponds to the following formula:



The balance comprises uncombined sodium formaldehyde sulfoxylate and sodium sulfate.[12] Diasone is sufficiently pure and uniform to be satisfactory in treating infected animals and human beings as well. This drug can be quantitatively estimated by diazotization and subsequent formation of a dye, according to a modification of a method of Bratton and Marshall.[13]

BIOLOGIC DATA ON DIASONE

Dissolved in distilled water, diasone was orally administered to fifty-two mice. As may be seen from Table I, 100 per cent of the animals which received 2 and even 3 Gm. of the drug per kilogram body weight survived. When 4 Gm. per kilogram were administered to each of fifty-six animals, 82 per cent survived. Of a group of thirty-six mice given 5 Gm. per kilogram 53 per cent survived. Several mice received 6 Gm. per kilogram, a dose which proved lethal. The conclusion may be drawn that for mice the toxicity of diasone is very low.

TABLE I

TOXICITY OF DIASONE IN AQUEOUS SOLUTION ADMINISTERED ORALLY TO MICE*

DOSE PER KILOGRAM (GM.)	NUMBER OF ANIMALS	SURVIVALS	
		NUMBER	PER CENT
2.0	18	18	100
3.0	34	34	100
4.0	56	46	82
5.0	36	19	53
6.0	5	0	0

*Each animal received a single dose.

The chronic toxicity of diasone was investigated on fifty mice to which the drug in food was administered for twenty-one consecutive days. Of these animals, 90 per cent survived a diet containing ¾ per cent of diasone (Table II). A diet containing ¾ per cent of sulfadiazine was tolerated by only 60 per cent, while a 1 per cent diet was lethal to all of ten animals.

TABLE II

CHRONIC TOXICITY OF DIASONE FED TO MICE FOR TWENTY-ONE DAYS

CONCENTRATION OF DRUG IN FOOD (PER CENT)	NUMBER OF ANIMALS	SURVIVING 7 DAYS		SURVIVING 14 DAYS		SURVIVING 21 DAYS	
		NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
¼	10	10	100	9	90	9	90
½	20	18	90	17	85	17	85
¾	10	10	100	10	100	9	90
1.0	10	8	80	7	70	6	60

Studies on twenty-five rats indicated that more than 7 Gm. of diasone per kilogram could be tolerated, a further manifestation of the drug's low toxicity (Table III).

TABLE III

TOXICITY OF DIASONE IN AQUEOUS SOLUTION ADMINISTERED ORALLY TO RATS

DOSE PER KILOGRAM (GM.)	NUMBER OF ANIMALS	SURVIVALS	
		NUMBER	PER CENT
3.0	5	5	100
4.0	5	5	100
5.0	5	5	100
6.0	5	5	100
7.0	5	5	100

Rabbits do not generally tolerate chemical compounds as well as do mice or rats. Experiments were performed on twenty-nine rabbits with the result that 100 per cent survived 2.5 Gm. per kilogram, while 83 per cent tolerated 3 Gm.

(Table IV). Eighty per cent of the animals withstood 3.5 Gm., but only 40 per cent could tolerate 4 Gm. of the drug orally. It appears that the maximum tolerated dose is therefore between 3 and 3.5 Gm. per kilogram.

TABLE IV

TOXICITY OF DIASONE ADMINISTERED ORALLY TO RABBITS

DOSE PER KILOGRAM (GM.)	NUMBER OF ANIMALS	SURVIVALS	
		NUMBER	PER CENT
1.0*	3	3	100
1.5	2	2	100
2.0	3	3	100
2.5	5	5	100
3.0	6	5	83
3.5	5	4	80
4.0	5	2	40

*Each dose was dissolved in 100 c.c. of water.

We were interested to note that rabbits also tolerated well an intravenous administration of diasone. Each of eighteen rabbits receiving 1 Gm. per kilogram intravenously survived without loss of weight for an average period of thirty days (Table V). An increase of the dose to 1.5 Gm. gave a 40 per cent survival and 2 Gm. a 33 per cent toleration. By contrast, sodium sulfadiazine could be given in only a ½ Gm. dose per kilogram. Larger doses were not tolerated.

TABLE V

TOXICITY OF DIASONE ADMINISTERED INTRAVENOUSLY TO RABBITS

DOSE PER KILOGRAM (GM.)	NUMBER OF ANIMALS	SURVIVALS	
		NUMBER	PER CENT
1.0	8	8	100
1.5	5	2	40
2.0	3	1	33

Drug given in a 10 per cent solution, containing 10 per cent sodium bicarbonate. The last-named substance was added for the purpose of holding the drug in solution. Otherwise in the presence of air the solution becomes oxidized and cloudy.

In conclusion, it may be stated that the chronic and acute toxicity of diasone tested orally and intravenously on mice, rats, and rabbits is far less than that of most well-known sulfonamides, including sulfadiazine.*

From Table VI it is readily seen that dogs tolerate diasone in large daily oral doses for long periods of time. By way of comparison, it should be noted that the dose found practical at present and generally used by clinicians investigating the treatment of tuberculosis in human beings is about 0.015 Gm. per kilogram (approximately 1 Gm. of diasone per day). For sixty consecutive

*F. T. Callomon was kind enough to make histopathologic studies which may shed some light on deaths occurring among mice when fed diasone. The following is quoted: "At ¼ per cent drug diet there were no changes from the normal except for some focal epithelial alteration in a few convoluted tubules with some hyaline casts in the tubules. At ½ per cent drug diet obvious epithelial damage was found in some dilated tubules, such as swelling and vacuolization of the cells which showed indistinct outlines, pale nuclei, and were partly anuclear. Hyaline casts and some leucocytic infiltration were observed. At ¾ per cent drug diet these changes became more obvious than at ½ per cent drug diet, for a greater part of the convoluted tubules, some of Henle's tubules, and also some collecting tubules were afflicted. Cellular and hyaline casts were found in many tubular spaces, while the capsular spaces were not obviously dilated, containing sometimes some hyaline precipitate. In the cortical district leucocytic infiltration was observed around or between some damaged, partly collapsed tubules. Spleens and livers of the mice were normal at ¼ per cent and ½ per cent drug diet, while cloudy swelling was observed in the livers, and a moderate reduction of the lymphocytes of the mantle zone with hyperplasia of germinal centers in the spleens of the mice . . . . neither in the mouse nor dog kidneys were gross concrements (uroliths) found and no corresponding destruction of the renal tissue."

TABLE VI

CHRONIC TOXICITY OF DIASONE ADMINISTERED ORALLY TO DOGS

DOG	DAILY DOSE (GM. PER KILOGRAM)	TOTAL DAILY DOSE	NUMBER OF ADMINISTRATIONS
1	0.050	0.850	60
2	0.050	0.725	60
3*	0.035	0.500	180
4	0.035	0.500	190
5	0.300	3.5	110
6†	0.300	3.5	100

No diarrhea or vomiting was observed in any of the animals.
*Gave birth to four puppies and raised them to mature state.
†Gave birth to eight puppies and raised them to mature state.

days, Dogs 1 and 2 received a daily dose of 0.05 Gm. per kilogram, which is three times larger than the daily human dose. These animals were sacrificed, and at autopsy the kidneys were seen to be only slightly affected. Dogs 3 and 4 received for 170 days a dose of 0.035 Gm. per kilogram per day, more than twice the therapeutic human dose. The animals did not lose weight and showed no signs of toxic effects. They were obviously in vigorous health and maintained a normal appetite. Dog 5 was given daily a large dose of 0.3 Gm. per kilogram, equivalent to twenty therapeutic human doses. The dog's original weight at the inception of the experiment was 22 pounds, but after ninety-five daily administrations of diasone it increased to 26 pounds. Concentrations of the drug in blood were estimated periodically four hours after the administration of the drug and were found to average 2 mg. of diasone per 100 c.c. of blood. Considerable quantities of diasone were found in the urine and lesser amounts in the feces. Dog 6 also received a daily dose of 0.3 Gm. per kilogram for eighty-three days. No diarrhea, vomiting, or other untoward symptoms were observed. The urine was negative for albumin and sugar, the weight was well maintained, and the health of the animal appeared good. It is interesting to notice that the prolonged administration of diasone did not unfavorably influence the course of pregnancy in Dogs 3 and 6. The first of these had a healthy litter of four, the second a litter of eight. The young were suckled by their mothers and grew into vigorous animals.

BLOOD LEVELS AND URINARY EXCRETION OF DIASONE

Blood levels were determined in mice that received food containing ½ per cent diasone for anywhere from four hours to six consecutive days. Since each of the animals had to be sacrificed in order to supply the necessary quantity of blood, different mice were used at various time intervals. The total blood levels ranged only from 2.0 to 3.8 mg. per 100 c.c. of blood (Table VII).

Mice that received orally only one dose of 0.01 Gm. of diasone (approximately 0.5 Gm. per kilogram) maintained fairly uniform blood levels during twenty-four hours, after they reached the highest point in the first six hours (Table VIII).

A rabbit whose oral intake of diasone was one dose of 1 Gm. per kilogram, or a total of 2.75 Gm., also showed a low but consistently uniform blood level during twenty-four hours. A low blood level, however, does not indicate an in-

TABLE VII

DETERMINATION OF BLOOD LEVELS IN MICE FED A DIET CONTAINING ½ PER CENT DIASONE

BLOOD EXAMINED*	MG. PER 100 C.C. BLOOD		
	FREE	CONJUGATED†	TOTAL
4 hr. after starting drug	1.31	Not tested	
4 hr. after starting drug	0.6	Not tested	
24 hr. after starting drug	1.32	0.68	2.0
24 hr. after starting drug	1.46	0.84	2.3
48 hr. after starting drug	3.1	0.7	3.8
48 hr. after starting drug	2.0	1.1	3.1
6 days after starting drug	1.63	1.27	2.9
6 days after starting drug	1.63	1.27	2.9

*Each experiment was performed on a different animal, sacrificed for the necessary amount of blood.

†Conjugated refers to results obtained upon boiling the blood filtrate with 20 per cent para-toluene sulfonic acid.

TABLE VIII

BLOOD LEVELS

DIASONE IN AQUEOUS SOLUTION ADMINISTERED ORALLY TO MICE
ONE DOSE OF 0.01 GM. PER KILOGRAM

BLOOD EXAMINED*	MG. PER 100 C.C. BLOOD (TOTAL)
2 hr. later	2.18†
2 hr. later	3.43†
6 hr. later	2.76
6 hr. later	2.19
18 hr. later	1.00
18 hr. later	2.18
24 hr. later	2.00
24 hr. later	1.64

*Each experiment was performed on a different animal, sacrificed for the necessary amount of blood.

†Conjugated not included.

complete absorption, since the urinary excretion of diasone reached 603 mg. in the twenty-four hour specimen and 324 mg. in the second-day specimen (Table IX). During the following nineteen days, the drug continued to be eliminated in the urine. After nineteen days, the total reached 1.194 Gm. Since we cannot account for a total of 2.75 Gm. of diasone ingested, it must be assumed that the drug was partially destroyed in the body or was changed into a chemical compound which could not be determined by the colorimetric method used. Comparatively small amounts were found in the feces.

Because of the large dosage, the highest blood levels were obtained in rabbits which received orally 0.5 Gm. per kilogram daily for a period of twenty days (Table X). The blood specimens were taken at two, four, and twenty or twenty-four hours after drug administration. The total blood levels (with one exception) ranged from 6.4 to 16 mg. per 100 c.c. shortly after drug intake, while at the end of the day the blood levels varied from 3.5 to 5.2 mg. In order to obtain higher blood levels, several rabbits received orally single doses of from 3.5 to 4 Gm. of diasone. As a result, the total blood levels ranged from 4.8 to 11.7 mg. per 100 c.c.*

Intravenous injection into a rabbit of a single dose of 0.2 Gm. per kilogram produced a moderately high blood level. After the first twenty-four hours

*The conjugated fraction of blood levels, following intravenous injections of diasone into rabbits, appears to be irregular, with a tendency to be higher in the first four hours.

TABLE IX

BLOOD LEVELS AND ELIMINATION IN URINE

DIASONE ADMINISTERED ORALLY TO A RABBIT—ONE SINGLE DOSE OF 1 GM. PER KILOGRAM*

TIME AFTER ADMINISTRATION	BLOOD (MG. IN 100 C.C.)			URINE (MG. IN 24-HOUR SPECIMEN)		
	FREE	CONJUGATED†	TOTAL	FREE	CONJUGATED†	TOTAL
2 hr.	1.15	0.65	1.80			
4 hr.	1.38	0.64	2.02			
24 hr.	1.52	1.15	2.67	439.57	163.83	603.40
48 hr.				256.28	67.60	324.48
3 days				62.56	21.62	84.18
4 days				29.58	18.91	48.49
5 days				25.20	6.80	32.00
6 days				11.18	11.52	22.70
7 days				7.6	2.15	9.75
8 days				2.06	2.84	4.90
9 days				2.97	1.08	4.05
10 days 11 days				9.75	4.16	13.91
12 days 13 days 14 days 15 days 16 days				27.47	18.87	46.34
17 days 18 days 19 days				Trace		
			Total	874.82	319.38	1194.20

*A total of 2.75 Gm. of diasone.

†Conjugated refers to results obtained upon boiling the blood filtrate with 20 per cent para-toluene sulfonic acid.

TABLE X

DETERMINATION OF BLOOD LEVELS IN RABBITS

DIASONE GIVEN ORALLY 0.5 GM. PER KILOGRAM DAILY FOR A PERIOD OF TWENTY DAYS

WEIGHT (GM.)	DATE	BLOOD TAKEN	BLOOD LEVELS (MG. PER 100 C.C.)		
			FREE	CONJUGATED*	TOTAL
First rabbit					
1,900	6/30/43	2 hr.	4.0	10.40	14.40
		6 hr.	5.25	3.40	8.65
		20 hr.	1.60	2.40	4.0
		After 15th dose			
1,580	7/ 8/43	2 hr.	1.52	5.28	6.80
		4 hr.	3.36	11.64	15.00
		24 hr.	3.40	1.08	4.48
		After 20th dose			
Second rabbit					
1,940	6/30/43	2 hr.	1.55	12.45	14.00
		4 hr.	1.90	14.10	16.00
		20 hr.	1.60	1.90	3.5
		After 15th dose			
1,840	7/ 8/43	2 hr.	1.6	1.40	3.0
		4 hr.	1.28	5.14	6.42
		24 hr.	1.02	4.18	5.20
		After 20th dose			

*Conjugated refers to results obtained upon boiling the blood filtrate with 20 per cent para-toluene sulfonic acid.

the urine of the same rabbit contained substantial quantities of diasone. During the six days following, only small quantities were excreted, with a final total of 272.4 mg. A very small amount was found in the feces. The amount excreted again did not equal the intake.

On the other hand, high blood levels, with no conjugated fraction present, were obtained when a larger dose of diasone amounting to 1 Gm. per kilogram was administered intravenously to a rabbit. These blood levels ran from 18.4 to 23.6 mg. per 100 c.c. The urinary excretion continued for eight days; it totaled 527.9 mg. of diasone, or 26 per cent of the amount administered.

When diasone was given orally to dogs in daily quantities of 0.035 Gm. per kilogram, the blood levels were found to be fairly low (from 0.7 to 1.2 mg.), and no conjugated fraction was present. When a larger daily dose of 0.3 Gm. per kilogram was administered, the blood levels reached 3.2 mg. and in one instance 7.8 mg.

The urinary excretion of diasone in dogs which received 0.035 Gm. per kilogram was found to be between 23.9 and 81.4 mg. in a twenty-four hour specimen (Table XI). On the other hand, the two other dogs whose intake of the product was considerably higher showed in their twenty-four hour urinary excretion from 107.3 to 544 mg. of the drug. It must be noted again that, as in the case of the rabbits, the quantities of the drug ingested were not commensurate with its elimination.

TABLE XI

URINARY EXCRETION OF DIASONE BY DOGS

DOSE	TIME AFTER ADMINISTRATION (HR.)	NUMBER OF DAYS AFTER START OF EXPERIMENT	URINE	FECES
			MG. IN 24-HR. SPECIMEN (FREE)	MG. IN 24-HOUR SPECIMEN (FREE)
0.035	24	103	81.42	55.82
0.035	24	106	39.13	30.60
	24	160	23.98	32.18
0.3	24	63	107.30	201.96
	24	112	544.00	75.95
0.3	24	64	117.9	143.6

Urine contained no albumin or sugar.

THERAPEUTIC EFFECTS OF DIASONE

In all of the therapeutic experiments, drugs were administered to mice in food according to the method described by Litchfield, White, and Marshall.[14] Mice were infected with the beta-hemolytic streptococcus strain C203, using 1,000 minimum lethal doses. By a lethal dose is meant one containing sufficient bacteria of a certain virulence which would eventually kill mice in the course of several days. It was necessary, therefore, to infect mice with 1,000 minimum lethal doses in order to kill the animals in the first twenty-four to forty-eight hours. From Table XII one can see, for example, that the ten control mice died on the first day. Those fed the ¼ per cent diasone diet showed a therapeutic effect superior to that exerted by a diet containing ¼ per cent sulfanilamide. But with a diet of ½ per cent sulfanilamide, the level at which the optimum effect of the sulfonamide is produced, animals show a better survival rate than with diasone.

TABLE XII

COMPARATIVE THERAPEUTIC EFFECTS OF DIASONE AND SULFANILAMIDE ON MICE INFECTED WITH BETA STREPTOCOCCUS HEMOLYTICUS STRAIN C203

PER CENT OF DRUG IN FOOD	DRUG	NUMBER OF MICE USED	PER CENT OF SURVIVALS DAYS 1	2	3	4	5	6	7	14	21	28
¼	Diasone	20	95	85	75	75	75	75	75	65	65	65
½		15	87	87	80	73	67	60	60	47	47	47
¼	Sulfanilamide	10	90	40	40	40	40	40	40	40	30	30
½		10	100	100	70	70	70	70	70	70	70	70
	Controls	10	10	0								

From Table VII one can see that in mice receiving ½ per cent diasone the average blood level is indicated as 2.83 mg. per 100 c.c. of blood.

There is no information available in literature concerning blood levels in animals receiving ¼ or ½ per cent sulfanilamide drug diet. It was found that blood concentration in mice given a 1 per cent sulfanilamide drug diet is 11.6 mg. per 100 c.c. (Feinstone and others: Bull. Johns Hopkins Hosp. **67**: 434, 1940.)

In our experiments on type II pneumococcus infection, we used strain M-48, as described in a previous publication.[15] Mice were infected intraperitoneally with ten minimum lethal doses containing about 250 bacteria. From Table XIII it may be noted that a diet containing ¼ per cent diasone had a considerably greater therapeutic effect than did a diet of ¼ per cent sulfadiazine. With a ½ per cent drug diet of sulfadiazine, however, the survivals were somewhat higher than in the case of diasone.

TABLE XIII

COMPARATIVE THERAPEUTIC EFFECTS OF DIAZONE AND SULFADIAZINE ON MICE INFECTED WITH PNEUMOCOCCUS TYPE II STRAIN M-48

PER CENT DRUG IN FOOD	DRUG	NUMBER OF MICE USED	PER CENT OF SURVIVALS DAYS 1	2	3	4	5	6	7	14	21	28
¼	Diasone	20	100	95	95	90	90	75	55	40	40	40
½		30	100	93	93	93	87	83	80	50	43	43
¼	Sulfadiazine	40	95	75	50	33	25	25	15	5	5	5
½		40	100	100	100	100	93	93	88	58	55	55
	Controls	15	27	0								

See footnote to Table XII for diasone blood level.

The average blood level of sulfadiazine administered to mice receiving ½ per cent drug in the diet was found to be 30.6 mg. per 100 c.c. (Feinstone and others: Bull. Johns Hopkins Hosp. **67**: 435, 1940.)

SUMMARY

In general conclusion it may be stated that the chronic and acute toxicity of diasone tested orally and intravenously on mice, rats, and rabbits is noticeably less than that of most well-known sulfonamides, including sulfadiazine. It is far less toxic than diamino-diphenyl-sulfone. Diasone has shown high therapeutic effectiveness in mice infected with beta-hemolytic streptococcus as well as type II pneumococcus infection. At the present time, diasone is being investigated clinically in the treatment of tuberculosis.

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POLYCYTHEMIA PRODUCED BY COBALT IN THE DUCK

A Hematologic and Pathologic Study

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COBALT polycythemia was first produced in rats in 1929 by Waltner and Waltner.[1] It has been produced also in rabbits,[2] dogs,[3] guinea pigs, mice, frogs,[4] and pigs.[5] This type of experimental polycythemia is accompanied by reticulocytosis,[2, 6-8] and an increase of blood volume.[9, 10] Hyperplasia of the bone marrow has been observed to occur in the dog and the rabbit.[11, 12]

The exact mechanism by which cobalt stimulates erythropoiesis is not known. It has been suggested by the Barrons[13] and by Davis[14] that the element cobalt may act upon the hemopoietic system by impairing cellular oxidations. Since exposure of animals to low atmospheric pressure,[15] daily physical exercise,[16] or continued administration of vasoconstrictor drugs[17] may induce polycythemia, it seems that the common denominator or the adequate stimulus to accelerated erythrocyte formation is probably hypoxia in the erythropoietic tissue.

It has been shown by one of us (R. H. R.) that a hyperplasia occurs in the extramedullary blood-forming tissue of the duck when this bird is infected with malaria.[18] It was suggested from this study that the hyperplasia is the response of the duck to the severe anemia which occurs in fowls with a high parasitemia. Since cobaltous chloride is known to produce a polycythemia in rabbits, dogs, guinea pigs, mice, frogs, and pigs, an experiment was outlined (1) to determine if polycythemia could be produced in the duck with cobalt and (2) to study in detail the hematologic and the pathologic lesions occurring in the duck and to compare these with the lesions observed in ducks infected with *Plasmodium lophurae*.

METHODS AND MATERIALS

The ducks used in these experiments were white Pekins, 3 weeks of age, varying in weight from 150 to 425 grams. They were kept together in the laboratory and fed Startena.* The birds were weighed at frequent intervals during the experiment.

Cobaltous chloride in a 2.0 per cent aqueous solution was given subcutaneously over the back. The ducks used in the first experiment were given once each day 1 mg. of cobalt per 100 Gm. of body weight up to a maximum dose of 4 mg. In the second experiment 4.0 mg. were given daily in a similar manner.

Red blood cell counts were made on blood drawn directly into diluting pipettes from a puncture of a vein in the webb. The diluting fluid was Hayem's solution. Counts were made each day on the birds included in the first experi-

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ment. Blood films were made from the webb on the seventh experimental day in the first experiment and in both experiments at the time of death of each duck. These were stained with Wright's modification of Leishman's Romanowsky stain. Counts were made on five or more oil immersion fields in different parts of each film until a minimum of 500 cells had been counted. Cells classed as immature were chosen on the basis of three criteria: nuclear structure, nuclear shape, and the extent of cytoplasmic chromation.

The ducks were sacrificed by clamping the necks. An autopsy was performed immediately thereafter and the tissues were placed in both Bouin's solution and a 10.0 per cent solution of formalin. Those fixed in the former preservative were used for histopathologic studies. The sections were stained with hematoxylin and eosin. Bone marrow for sectioning was obtained from the femur.

Imprint (dab) films were made at the time of autopsy from the liver, spleen, rib, and the femur. These were stained with Wright's modification of Leishman's Romanowsky stain. They were examined under oil immersion, care being given in those of the liver and spleen, particularly, to note evidence of erythropoiesis. Counts were made of the phagocytic cells present and an estimate was made of the amount of phagocytosed material they contained in each case. Films of bone marrow were studied carefully for evidence of changes in plasticity in the erythrocytic series.

The number of ducks used and the time that each experimental procedure was carried out is given in the different experiments.

EXPERIMENTS

Experiment 1.—Six ducks, 3 weeks of age, were used. Four were given cobalt and two were kept as the controls. The total number of red blood cells and the time of the injection of the cobalt are given in Fig. 1. These birds were killed on the fourteenth day following the first injection of cobalt. The erythrocyte numbers increased by 22 to 38 per cent in the ducks given cobalt (Fig. 1). No significant variation occurred in the number of red blood cells in the two control birds during this same interval.

Experiment 2.—Fifteen ducks, 3 weeks of age, were used. Ten were given 4 mg. of cobalt daily, while five birds were kept as controls. On the third day of the experiment, three of the ducks given cobalt and one untreated bird were killed; on the seventh experimental day three ducks given cobalt and one control were killed; on the nineteenth experimental day two ducks given cobalt and one control were killed; on the twenty-eighth day of the experiment the remaining two cobalt-treated ducks and two controls were killed.

Fig. 2 gives the average number of red cells for the second group of ducks at each time they were counted. The red blood cells increased in the ducks given cobalt, as shown in Fig. 2, until about the eleventh day; from this time until the twenty-eighth day the total number of red cells remained approximately constant. The number of cells during this interval was increased by 38.0 per cent over the number present at the beginning of the experiment. The average number of erythrocytes for the control birds remained practically constant for the first eleven days of the experiment. At this time the number of

red cells increased, reaching their maximum number on the twenty-first day of the experiment, when there was an increase of 15 per cent in the number of red cells of the control animals as compared with the number at the beginning of the experiment.

The average weight of the birds in the control and the experimental groups is given in Fig. 2. There was a progressive increase in the weight of all birds. The average increase of those in each group was approximately the same.

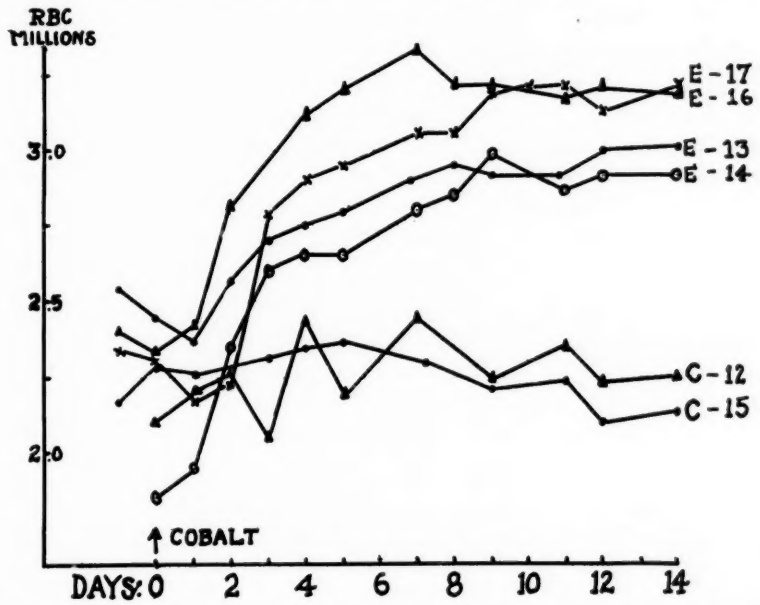

Fig. 1.—The effect of daily injections of cobaltous chloride on the red blood cell counts of young ducks. *E*, Experimental cobalt-treated ducks. *C*, Control ducks, untreated.

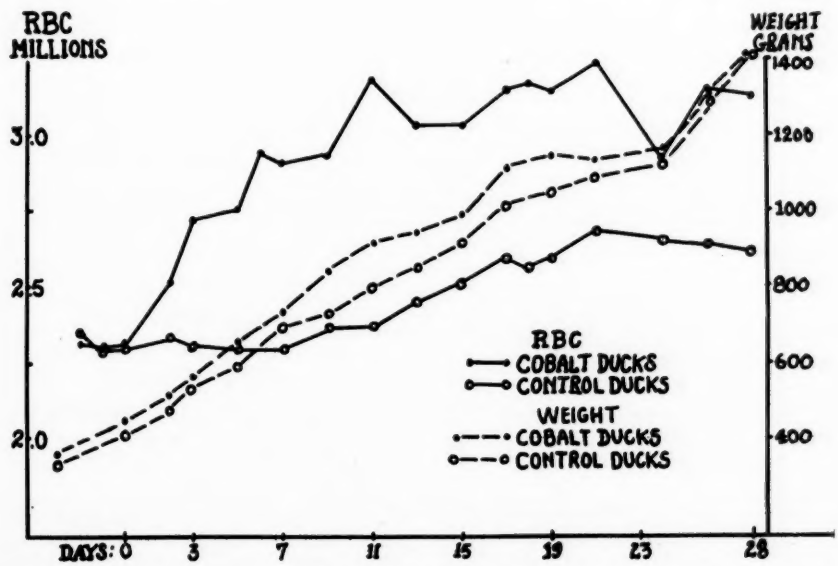

Fig. 2.—Average erythrocyte numbers and body weights of a cobalt-treated group and a control group of ducks. At the start of the experiment the cobalt-treated group consisted of ten ducks and the control group of five ducks.

HEMATOLOGIC STUDIES

Blood Films.—In Table I are given the number of cells counted and the percentages of immature cells found, arranged in terms of experimental days. With the exception of Duck 13, the general trend is toward an initial rise in the percentages of young red cells during the first three days of cobalt injection, followed by a continuous decline in these percentages with continued medication. The control animals showed some decline but much less than did the experimental ducks. With the fall in young cell percentages in the experimental animals, there was increasing nuclear degeneration and pyknosis, cellular fragmentation, and the appearance of cells with highly immature nuclei and cytoplasm showing primitive basophilia. These latter changes were not found to accompany the slight decrease in young cell per centage in the control birds.

Films From Liver and Spleen.—Films made from the liver and spleen gave data which were supplementary with one exception. They showed the extent of phagocytic activity, and in one case there were signs of extramedullary erythropoiesis in the liver.

These preparations in both groups showed more normal, mature red cells in the experimental than in the control animals. While this is a subjective estimate, it may indicate that these organs in the experimental animals were more congested with blood.

TABLE I

PERCENTAGES OF YOUNG ERYTHROCYTES IN SMEARS OF PERIPHERAL BLOOD

EXPERIMENTAL DAYS	DUCK	GIVEN COBALT CHLORIDE	TOTAL NUMBER OF CELLS COUNTED	PERCENTAGE OF IMMATURE CELLS	REMARKS
3	111	+	884	7.2	Some primitive nuclei
	114	+	692	6.8	Some primitive nuclei
	119	+	732	8.0	A few atypical cells
	123	0	902	6.2	
7	12	0	1,210	6.6	
	13	+	659	5.8	Pyknosis, degeneration
	14	+	668	5.7	
	15	0	852	6.7	
	16	+	686	4.0	Much degeneration
	17	+	820	5.6	Some degeneration
	115	+	788	3.1	Much cellular debris
	121	0	677	6.0	
	117	+	574	2.9	Many primitive nuclei
	116	+	719	3.4	A few polychromatophils
14	12	0	920	8.1	
	13	+	963	28.4	Extreme fragmentation; many very primitive cells, some showing pyknosis
	14	+	505	2.0	Pyknosis
	15	0	620	9.9	
	16	+	681	3.5	Degeneration
	17	+	780	2.8	Nuclear degeneration; no polychromatophils
19	112	+	729	2.2	Much fragmentation
	122	0	602	5.8	
	113	+	612	2.8	Very few polychromatophils
28	109	+	1,024	1.6	Very much cellular debris
	110	+	824	3.0	Many atypical cells
	120	0	712	5.1	Young cells primitive
	124	0	698	5.7	

Phagocytic cells in both hepatic and splenic smears from birds killed on the third and seventh days were slightly more numerous in the experimental animals than in the controls. A striking observation, however, was the much smaller amount of phagocytosed material in the cells of the experimental birds which were killed on the fourteenth, nineteenth, or twenty-eighth days. The difference was too great to be accounted for by the slight increase in number of cells and is particularly striking in the light of the much greater amount of debris found in peripheral blood of these experimental birds.

In Duck 13, killed on the fourteenth day of the first experiment, there were found, in the imprint smear of the liver, numerous erythroblasts which were primitive enough to show no polychromatophilia. Since these cells were not found in the peripheral circulation, it is assumed that their presence in the liver indicates some hepatic erythropoiesis in this bird. It will be recalled that this bird is the one that, at time of autopsy, showed an extremely elevated percentage of immature cells (Table I).

Marrow Preparations.—In all birds, films were made from both rib and leg marrow. Findings in every case differed slightly in the rib and leg marrow from the same bird. In the controls, there was evidence of slightly more active erythropoiesis in the rib marrow. In the experimental animals the rib marrow showed changes more pronouncedly than did the leg marrow, but, after the fourteenth day, these changes were progressively hypoplastic.

In the first group of birds which was killed on the fourteenth day, the rib and leg marrow of the experimental animals showed some stimulation of red cell production with only one essential difference; that of the leg showed a mild, typical hyperplasia; that of the rib showed an increase in more mature cells of the erythropoietic series but was impoverished in the basophilic and early polychromatophilic erythroblasts. This difference was noted also in the leg marrow of Duck 13, where the hyperplasia of the late erythropoietic elements was very great, but the early stages were entirely absent.

In the second experiment the changes described occurred progressively in the experimental animals killed on the nineteenth and twenty-eighth days. In each bird the changes noted were more advanced in the rib as compared to the leg marrow. The rib marrow of Ducks 109 and 110, killed on the twenty-eighth day, showed a generalized hypoplasia of the erythropoietic series which seemed particularly advanced in Duck 109. The leg marrow reflected the same change clearly, but in both birds there was an indication of plasticity confined largely to the acidophilic erythroblasts.

PATHOLOGIC STUDIES

Each of the ducks appeared normal at the time it was sacrificed. The skin over the back was desquamated at the site of the injections and the underlying subcutaneous tissue frequently was indurated. The pathologic lesions here were those of an acute and chronic reaction.

No gross changes were observed in the organs of any of the ducks. The weight of the organs of the birds in the experimental group did not vary significantly from the controls. The femur bone marrow from the ducks given cobalt for three-, seven-, and fourteen-day periods was more red in color and

semiliquid when compared to that of the corresponding controls. The marrow sections from the ducks injected with cobalt for twenty-eight days did not show microscopically a significant difference from that of the controls (Fig. 3). In the hyperplastic bone marrow, the predominating cell was large and mononuclear; the nucleus was usually either round or oval. The nucleus stained lightly with

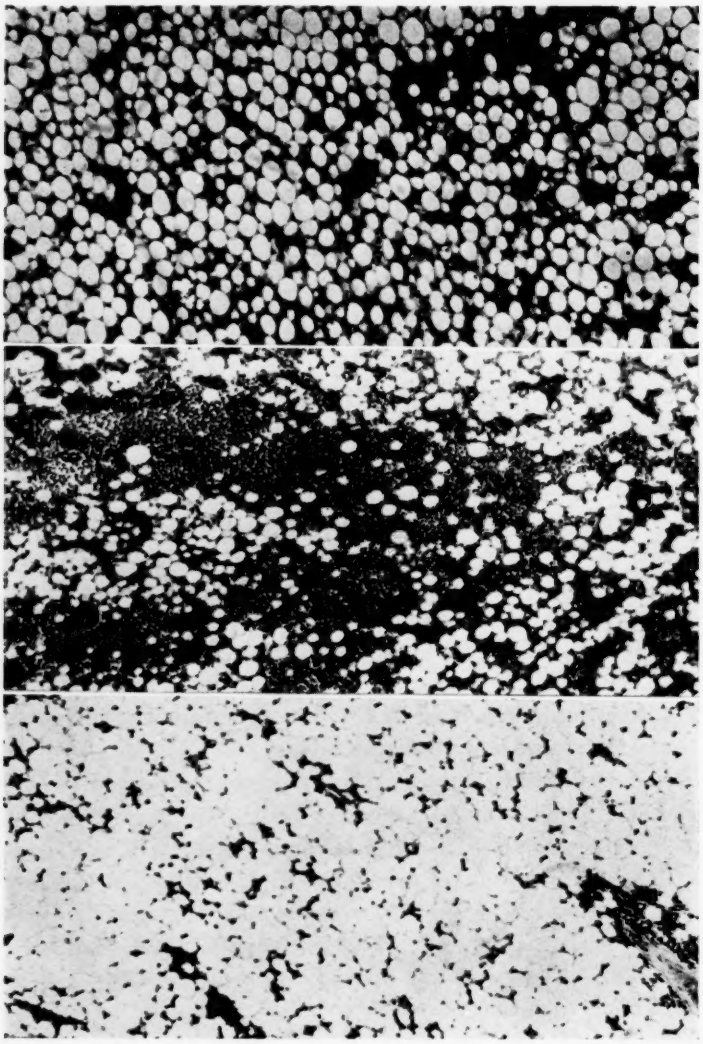

Fig. 3.—*A*, Femur marrow from Duck 121. This is a normal duck and is the control for Duck 116 shown in *B*. *B*, Femur marrow from Duck 116 given cobalt for seven days. The marrow is hyperplastic. *C*, Femur marrow from Duck 110 given cobalt for twenty-eight days. The marrow is hypoplastic.

hematoxylin in some of the sections, while in others it stained deeply. The cytoplasm varied in its staining reaction from a light pink to a deep red. In some of the birds the cytoplasm of these marrow cells was filled with large red particles.

There was an increase in the number of cells in the masses of extramedullary hemopoietic tissue in the sections of lungs, liver, adrenals, and kidney from the

ducks given cobalt for the three-, seven-, and fourteen-day periods. The control ducks of corresponding age showed few, if any, extramedullary foci of blood-forming tissue in the liver and kidney. The experimental ducks had large foci of these cells protruding into the lumina of the blood sinuses (Figs. 4 and 5). These cells were mononuclear and dark staining. In the lumina of some of the large blood vessels, groups of cells were present similar to those forming the masses projecting into the lumen. It was obvious from microscopic study that

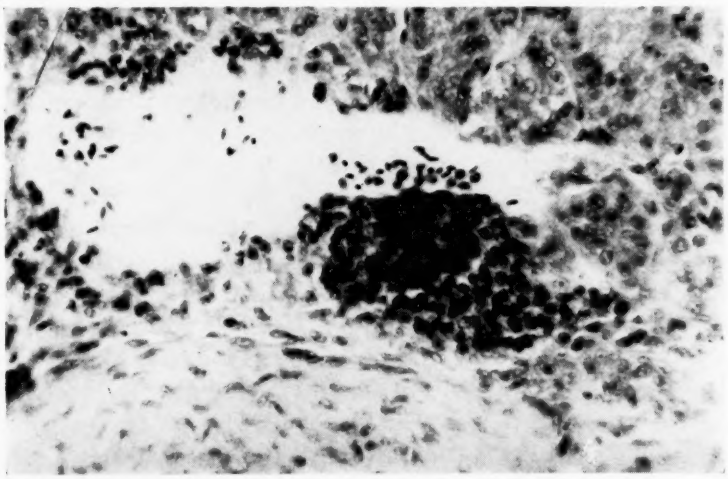

Fig. 4.—Hyperplasia occurs in the extramedullary erythroblastic tissue in the ducks given cobalt for a short period. The adrenal from Duck 111 given cobalt for three days. Note the projection of these cells into the lumen of the blood vessel.

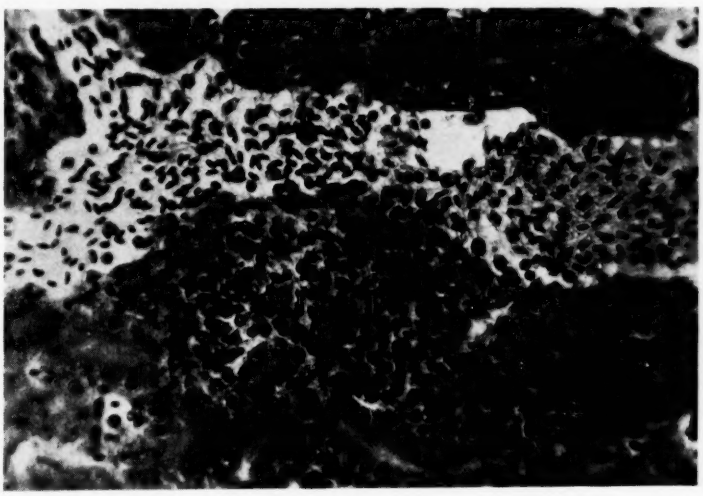

Fig. 5.—Hyperplasia occurs in the extramedullary erythroblastic tissue in the kidney similar to that in the adrenal. Duck 114 was given cobalt for three days.

these were neither agglutinated red cells nor groups of normal circulating white blood cells.

There was an apparent increase in the number of cells in the splenic pulp of ducks given cobalt. The increase occurred in the number of large mononuclear cells, many of which had eosinophilic particles in their cytoplasm. Hyperplastic

masses of these reticular-like cells projected into the lumen of the splenic sinuses in a manner similar to those in the liver, kidney, and adrenal.

DISCUSSION

Polycythemia may be produced in the duck following the subcutaneous injection of cobaltous chloride. The increase in the number of cells in the erythroblastic series is similar to that occurring in mammals. As far as we know, this is the first time results have been published in which birds have been used for the study of polycythemia produced by cobalt.

In this experiment the increase in the total number of red cells rapidly progressed until the fourteenth day. From this time until the end of the experiment on the twenty-eighth day the number of red cells remained approximately constant. The number of cells during this interval remained at about 38 per cent over that at the beginning of the experiment. It is of interest to observe that the red cells of the controls increased about 16 per cent during the same period. These observations suggest that the normal erythrocyte count for young ducks gradually increases with age from 3 to 6 weeks. The average number of red cells for 3-week-old ducks is 2.3 million. Hewitt observed a variation in the total number of red blood cells in young and adult ducks.[19]

The percentage of young erythrocytes is increased in the peripheral blood during the first few days following the initial injections of cobalt. A number of these young cells were found in a dab smear of the liver of Duck 13 on the fourteenth experimental day. The histologic studies of the tissues corroborate the hematologic observations of hyperactivity of the erythroblastic series. The bone marrow from the femur was hyperplastic in the ducks killed on or before the fourteenth day of the experiment. The extramedullary foci in the spleen, liver, adrenal, and kidney also showed a greater number of cells during this same period as compared with the control birds. It appears, therefore, that all the erythroblastic foci are stimulated within three days following the commencement of the subcutaneous injections of cobalt.

The ducks which were given cobalt showed a sustained polycythemia from the fourteenth day to the end of the experiment on the twenty-eighth day. During this time there was a gradual diminution in the number of young red blood cells in the peripheral blood. The femur marrow from the ducks given cobalt and sacrificed on either the nineteenth or twenty-eighth day of the experiment appeared the same in histologic sections as those of the controls of similar age. It was definitely hypoplastic as compared with the femur marrow from the birds given cobalt and sacrificed on the third, seventh, or fourteenth day. Furthermore, the foci of extramedullary erythropoiesis were absent in the ducks given cobalt and sacrificed on the nineteenth and the twenty-eighth days of the experiment. The hematologic and pathologic observations indicate that cobalt at first stimulates erythropoiesis, after which it may act either as a depressor or an injurious agent to the erythroblastic series.

Some consideration must be given the fact that total red cell counts persisted while the percentages of young cells were falling and the marrow showed an increasing hypoplasia. The mechanism of cobalt polycythemia has been studied by several workers. According to Orten, the general opinion seems to

be that in rats, rabbits and dogs, this polycythemia is a product of increased blood production rather than of decreased blood destruction.[7] In our animals, this stimulation of cell production would seem to be a feature of the early stages of cobalt administration. With the continuation of cobalt, however, there is a fall in erythropoietic activity not reflected in the total red cell counts of the peripheral blood. At the same time that the production of young cells is depressed, there is a depression in the phagocytic activity, or capacity, of the reticulo-endothelial elements of the spleen, particularly, and of the liver. At least this appears to be the case if one may estimate the phagocytic activity of such elements by the amount of phagocytosed pigment and cellular debris seen in their cytoplasm. It is suggested, therefore, that in ducks the initial rise in peripheral counts is a product of erythropoietic stimulation but that the maintenance of the elevated count may, in part, be the product of a depressed rate of blood destruction. Several observations of our own and those of others seem to bear out such a conclusion.

Anderson and co-workers[20] have found that a high blood-building diet is necessary to the maintenance of cobalt polycythemia in the rat. This fact, considered with our findings, may suggest that cobalt, in the absence of such dietary support, ultimately suppresses erythropoiesis. Frost and associates[21] found cobalt to have what they considered a toxic effect on young dogs, an effect that was partially compensated by the simultaneous feeding of whole liver. In the blood films of our ducks killed on the nineteenth and twenty-eighth experimental days there was evidence of increasing intravascular cellular fragmentation which, in association with the increasing marrow hypoplasia, may indicate a toxic process under way in these animals. If this interpretation is correct, it becomes necessary to assume some depression in rate of blood destruction to account for the maintenance of elevated erythrocyte counts. To support this assumption, there is the observation that at the time the greatest amount of cellular debris occurred in the peripheral blood, there was also the least phagocytosed material seen in the reticulo-endothelial elements, which one might normally expect to be loaded with it. These facts lead one to assume some depression in the blood-destroying activity in the duck as a consequence of continued cobalt administration.

To summarize the hematologic and pathologic data in the ducks given cobalt, it may be said that there occurs a period in the latter part of the experiment in which there is a marked increase in the total number of red blood cells, but the percentage of young cells has decreased and the number of erythroblastic cells in the bone marrow has decreased also. Furthermore, the erythrocytes at this time show cytologic evidence of injury. There also is evidence at this time of decreased phagocytic activity of the cells in the reticulo-endothelial system. There is, therefore, a polycythemia with cytologic and pathologic evidence of injury to the erythroblastic series.

Ducks infected with *Plasmodium lophurae* show a hyperactivity of the cells in the femur marrow and also of those in the extramedullary erythropoietic foci.[18] It has been suggested that this hyperplasia was the response of the birds to the acute and rapid loss of red blood cells produced by the malarial parasite. Similar histologic changes are present in the ducks given cobalt and killed between the third and fourteenth day. In malarial infected ducks there is

an anemia; in cobalt-treated birds there is a polycythemia. In each experiment there has been a stimulation of the hematopoietic tissue. These observations in the cobalt-treated ducks, therefore, apparently support the opinion of one of us (R. H. R.), that certain of the pathologic changes in malaria result from the anemia and are not specific for this disease.

CONCLUSIONS

1. Polycythemia has been produced in young ducks by subcutaneous injection of cobaltous chloride in aqueous solution.

2. This polycythemia is accompanied by early marrow hyperplasia and an increase in extramedullary erythropoietic masses in spleen, liver, kidney, and suprarenal.

3. With continued injection of cobaltous chloride, there is evidence of marrow hypoplasia, disappearance of extramedullary erythropoietic foci, and a lowered phagocytic activity of the reticulo-endothelial elements of the spleen and the liver.

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Presidential Address

LOOKING FORWARD TO SOME OF THE PROBLEMS OF MEDICAL EDUCATION IN THE POSTWAR YEARS

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CLINICAL research and graduate medical education are so closely interwoven in the modern fabric of medicine that societies which seek to advance the former should, by corollary, interest themselves deeply in the latter. It may not be amiss at this time, therefore, to bring to your notice certain imminent problems of this domain. These problems are held to be of particular importance at this time, as there can be no doubt about the coming strain in graduate and postgraduate medical education nor any question about the need for immediate, careful planning to meet the strain. A recent sampling of the postwar plans of medical men in the Armed Services, as conducted by the Council on Education and Hospitals of the American Medical Association, indicates that the great majority intend to obtain additional training of one type or another before entering or resuming the practice of medicine. The preliminary results of a survey now being conducted by the Minnesota State Medical Association are shown in Table I. This reveals clearly the high percentage of individuals who intend to pursue further study rather than to enter practice. While much the larger number want short courses, it is evident that many more want residencies than there are places available, at least in Minnesota institutions as at present organized. Many of you, I am sure, are receiving letters from young medical officers in various parts of the world who are trying to assure themselves of appointments as residents or assistant residents after the war. Personally, I have found it very difficult to answer these letters. One dislikes to disappoint men who are more deserving in seeking advanced education than any who have sought it in many generations; yet the problem is still so vague from a quantitative standpoint that nothing definite can be promised. The time of availability for the individual is unknown, even after the war, and, for the mass, it will undoubtedly be spread out over a period of years. This factor in itself will serve to lessen somewhat the acuity of congestion, regrettable though it may be for many of

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TABLE 1

QUESTIONNAIRE—INTENTIONS OF 500 MINNESOTA PHYSICIANS IN ARMED SERVICES*

Entering service from internship	68
Will enter practice	1
Want short courses	8
Want residencies	59
Entering service from residency	50
Will practice specialty	6
Want short courses	15
Want residencies	29
Entering service from practice	382
Re-entering general practice	31
Re-entering specialty practice	57
Want short courses	200
Want residencies	87
Remain in government service	7

*These data were provided through the courtesy of Mr. R. R. Rosell, Secretary, Minnesota State Medical Association, Medical Arts Bldg., St. Paul, Minn.

those in Service who would like to return to civilian life immediately upon the war's end. There is every reason to anticipate that the plotted curve of increasing participation in graduate medical eduction in the postwar years will be plateaulike rather than sharp peaked, comparable, if you like, to the pulse wave of aortic stenosis rather than to that of aortic regurgitation. It is highly probable that the wave of increase will be sustained for at least ten years, and perhaps indefinitely. Recognizing the obvious cause of the ascending portion of the curve as being due to the men gradually returning from Service, the question may well be asked as to what factors are going to maintain the plateau. One is ever more impressed that the old order changeth, yielding place to new, which in this instance is the crescendo increase of group practice and specialization in smaller communities. There appears to be but little doubt that the general practitioner of the future is going to be steadily more of an internist and less of a surgeon; a family physician and counselor who has been trained in the recognition of disease but who has no illusions about his ability to treat it surgically; one who is at least reasonably competent in medical management and at the same time aware of its limitations. In years gone by the general practitioner, in the truest sense of the term, was essential because of the factors of time and distance. Modern transportation is rapidly bringing these factors to the vanishing point. Excluding the temporary difficulties imposed by war, it will seldom be necessary any longer for a patient to be transported for more than a very brief period in order to have an emergency or complex problem of whatever nature cared for by a thoroughly competent specialist. There is little doubt that this trend will continue to develop after the war. We can look forward to a steady increase in the number of strategically located, well-equipped and well-staffed hospitals in smaller cities. Concrete plans along these lines are being contemplated in several states and, as many of you know, the Commonwealth Foundation has initiated a plan of this type in the South during recent years. All of these hospitals will undoubtedly be small centers of group practice requiring varying numbers of medical and surgical specialists.

Thus it seems evident that we are going to have the demand and supply for a sustained plateau in graduate medical education. This will require increased teaching material and staff. In many of the centers of medical educa-

tion the clinical material will suffice if it is made available in the proper way. Some universities, as, for example, those in Boston, have been able to avail themselves fully of the clinical material in their cities, to the great advantage of medical education, clinical research, and the care of the sick. In many other centers, however, a vast amount of first-class clinical material is not being employed to the greatest good. One additional question regarding available clinical material which looms large at the present time is that of the future relationship of the Veterans' Hospitals to medical education. It is generally understood that between three and four hundred thousand beds will be available for veterans throughout the United States. Obviously, a large number of trained specialists will be needed to provide adequate medical care in these institutions, and this need might well have been added to those factors mentioned a few moments ago which are believed likely to maintain the plateau of increase in graduate medical education. One must ask, however, to what extent a considerable segregation of the national clinical material in the Veterans' Hospitals might impose serious limitations upon the universities and teaching hospitals. I shall risk being thought naive to venture the belief that an affiliation of medical schools and Veterans' Hospitals for the purpose of teaching and research would appear to have many mutual advantages. In the past the Veterans' Administration has undoubtedly had to give thought to objections on the part of some to their being used for this purpose. This objection would be removed, however, if the arrangement were worked out on a purely voluntary basis. It is true that the location of many of the hospitals would preclude their use, but in the Twin Cities area, for example, a 500-bed hospital is near at hand. This hospital would provide excellent clinical material not otherwise available, as, for example, in Tropical Medicine.

In contemplating a program for a much larger number of graduates, it is evident that a broader base must be achieved and that this can be effected, with undoubted advantage to the individual, through greater diversification of training. It is clear that the direction which this pursues should depend to some extent upon whether the individual's goal is one of the practice of medicine, after certification of the Board, or whether, mainly, one of clinical teaching and research. In the former case, the plan should place more emphasis upon clinical medicine, while in the latter, somewhat more time should be devoted to the preclinical sciences and to experimental medicine. By this, I do not mean to imply that the individual can decide forthwith that he is going to pursue an academic career in medicine. He may have this plan and he may, indeed, have gauged his talents and abilities properly; on the other hand, it may become apparent after a year or so of exploration that he is better suited to the art and science of the practice of medicine than to the science and art of clinical investigation. Thus it is clear that an allowance of time will be necessary at the outset which should take the form of a period of training of equal value in either direction. With these reflections in mind, a program of the general type given in Table II is under tentative consideration.

It is obvious that each individual rotating through this schedule will not have service in all of the departments mentioned. Further, it is certain that some will drop out before the four years are completed, mainly because of real

TABLE II

First Year
6 to 9 months' assistant residency in medicine.
3 to 6 months' service in one or two minor fields: pathology, medical gynecology, clinical laboratory medicine, radiology
Second and Third Years
Same plan as in first year plus 6 months (total) as clinical assistant with university preceptor (private practice)
Fourth Year
6 to 9 months' residency in medicine
3 to 6 months in a minor subject, as noted in first-year plan

or imagined financial stringencies. Let us return to this problem in a few moments. The plan is sufficiently elastic to permit more emphasis on clinical training for those who are clearly suited for the practice of medicine and more on the fundamental sciences and on clinical research for those who show promise in the latter direction. Except for periods spent with the preceptor, the individual will be given teaching responsibility throughout, not only to gauge his aptitude for teaching, but also to provide an additional stimulus to the quest for knowledge. He will also be encouraged from the outset to devote whatever unused time his program may permit to a continuing research on a specific problem in medicine.

It may be noted that the clinical assistantship with a university preceptor is assigned to the second and third years. Where it is evident early that a man is best suited for practice, this period will be deferred until the third year. If the individual shows some promise for teaching and research but is not certain of his inclination, the period of contact with the practicing physician may be provided in the second year in order that he may have an earlier opportunity to make due comparison. A number of advantages are envisioned in such preceptorships, among which a better insight into the physician-private patient relationship may be stressed; that is to say, a superior opportunity of studying the art of medicine as compared with that usually available in the teaching hospital. It is recognized, of course, that difficulties will be encountered unless the greatest care is exercised in the choice of these preceptors and in the arrangements made between them and the university at the outset. It is believed, however, that the selection of a few outstanding men will gradually permit the establishment of a tradition of the utmost value. By means of these preceptorships, plus fellowships in certain hospitals not yet included in the graduate program, it is believed that the Department of Medicine of the University of Minnesota would be able to achieve a threefold expansion of its graduate enrollment.

A four-year program of the type just alluded to at once brings up one of the most important problems of graduate education: namely, the ever-increasing age at which an individual completes his formal training. This problem is the inevitable sequel to the need for more time to impart the expanding knowledge of the sciences. Increasing specialization in medicine has served, here, to prevent the problem from attaining impossible dimensions, but I think we are all aware of the economic and social difficulties often imposed upon the young man intent upon securing graduate instruction but unwilling to wait until he is 30 years of age or older before assuming family responsibilities. Not a few have existed penuriously for several years rather than forego either training or family. What measures might be taken to alleviate this difficulty at least in

part? For one thing, a graduated increase in stipend might be provided, depending upon the size of the individual's family, according to the established and thoroughly satisfactory method in use for government employees in certain countries. In this country we are constantly deploring the relatively low birth rate at the so-called "intelligentsia" level, yet our present salary scale in graduate education certainly counterbalances any propaganda which might encourage graduate students to have families, or larger families. If additional subsidies become available for advanced study after the war, some thought might well be given to the establishment of a sliding scale according to the number of dependents.

Another factor of perhaps more importance to the problem of late age of completion is that of the amount of time spent in primary education. As a nation, we have long been wedded to the concept that everyone, with exception of the occasional infant prodigy, must spend eight years in grade school and four in high school before entering college. One is reminded of the old verse of Persius*: "He still is moist and yielding clay; now, now, ere he congeal, we must make haste and tirelessly shape him on the sharp wheel."

We are just beginning to realize that the ability of school children and their need of a longer or shorter period of primary schooling before entering college must be treated in part, as a problem in bio-statistics. If 18 is the correct average age for college entrance, it is a statistical certainty that there are many who can enter at 17, quite a few at 16, and a fair number even below this age. Conversely, there are many who are not ready at 18, 19, or 20, and of this group the vast majority are not suited for a college education at all. In the past there has been a strong prejudice against acceleration of the primary education in individual cases because of the fear of psychologic maladjustments due to age disparity. There is an increasing feeling, however, that this difficulty can be avoided by an early and careful selection of groups or units which are advanced through the primary schools at varying rates, according to scholastic ability and other factors. I shall not attempt to discuss the difficulties of selection which such a proposal would entail, except to say that many leading educators now regard this general type of selection as entirely feasible on the basis of objective testing. Clinical investigators and teachers, in particular, ought to be vitally interested in this whole problem.

I must yield to the temptation to digress for a moment longer on the topic of primary education. May I recommend to any of you who have not already read it the charming essay of Montaigne on the "Education of Children," first published in 1580. In this, as translated by Florio, Montaigne says†: "Those which according to our common fashion undertake with one selfe-same lesson, and like maner of education to direct many spirits of divers formes and different humours, it is no marvell if among a multitude of children, they scarse meet with two or three that reap any good fruit by their discipline, or that may come to any perfection." This may be unduly severe for the twentieth century, but it is adequate evidence, nevertheless, that the concept which has just been upheld, that is, recognizing individual variations in intelligence and aptitude and teaching accordingly, is by no means a recent one.

*From *Selected Essays of Montaigne* translated by Donald Frame, published by Walter J. Black, New York, N. Y., for the Classics Club, 1944.

†Published by the Modern Library, Random House, Inc., New York, N. Y.

The accelerated curriculum which the medical schools of this country have adopted during the period of the war should be reconsidered from the viewpoint just presented. There are some medical students who will perhaps not suffer because of the acceleration and who will thus gain time for graduate education. On the other hand, there are many, probably a majority, who should have more time for study and contemplation and for whom the accelerated curriculum is not well suited. I am fully aware of the difficulty which a double curriculum would impose on the administration and faculty of any medical school. I do not offer any solution of this problem but simply wish to emphasize its importance.

Up to the present, the discussion has related in the main to graduate rather than postgraduate education. The war will really draw the distinction between these two to the vanishing point, mainly because of the difficulty which will certainly be encountered in classifying an individual's medical experience during his period of Service. Some will have gone from their brief internships to an Army or Navy hospital where they will have served for two to three years, often under careful supervision and with excellent clinical material and facilities. This group will have gained considerable experience of real value to their future career and may, therefore, have been considered to have had some of their graduate training. Others, however, will have gained little but a broader experience in living, and will, in fact, have lost much of what medicine they learned during their accelerated medical school curriculum. Undoubtedly, the best thing for this group, initially, would be a further period of internship, and it is to be hoped that this can be arranged in many instances.

In addition to those who went from their internship directly into the Armed Services, there will be a large number of men who had been in practice for from a few months to a number of years. Quite a few of the latter will want "refresher" courses lasting from two to six months, while many of the younger men will be willing to devote a year to postgraduate work before returning to practice. The lack of uniformity in this whole group is bound to make for the greatest difficulties both in policy and organization of courses. How can the most training be given to the individuals who are most in need of it? Obviously, a man who has had but nine months rotating internship preceding his Military Service can go directly into practice if he so elects but, just as obviously, to the probable detriment of medical care. Is this individual to be admitted to refresher courses of from six to eight weeks in length? The difficulty is, that if denied this privilege, some few will simply forego the longer courses which may be offered and enter practice sans refreshment. It is certain that this situation will have to be accepted in some measure as one of the inevitable backward journeys incident to war and that the best we can do is to minimize it by trying to induce all of the younger veterans to spend at least six months in an intensive postgraduate curriculum before entering practice.

The character of the postgraduate curriculum offered in any given center will, of necessity, vary with the available clinical material and extra teaching staff. Government or foundation subsidies of one source or another will be essential with rare exception. An attempt to plan a generally applicable curriculum would appear to have little value, but there are, nevertheless, certain guiding principles which we have accepted in formulating our own plans. The

first is to avoid short, purely didactic refresher courses. Eight weeks of intensive clinical instruction is believed to be the minimum length of course which should be planned, and younger men with little experience should be dissuaded from enrolling in the shorter courses and at the same time encouraged to take longer ones.

According to a tentative plan recently formulated* at the University of Minnesota, postgraduate instruction in medicine will be divided into two parts, as shown in Table III.

TABLE III

POSTGRADUATE PLAN

A. One-week survey courses in each of following:

Infectious disease	Radiology	Surgical diagnosis
Respiratory disease	Dermatology	Proctology
Cardiology	Neurology	Genitourinary diseases
Gastroenterology	Psychiatry	Orthopedics
Liver, biliary tract, and pancreas	Medical gynecology	Physical medicine
Hematology	Obstetrics	Anesthesia
Endocrinology—Metabolism	Pediatrics	Physiology
Pathology	Medical otolaryngology	
	Medical ophthalmology	

Limited to 30 individuals at one time

B. Six months to one year as volunteer or junior attending staff man rotating on various services

Limited to 10 individuals at one time

As noted in Section A, there are three units each of eight weeks' duration, or six months in all. It is planned to require registration for at least one eight-week unit, with the understanding that individuals may drop out and return at a later date for another unit if so desired. It is also planned that at least one eight-week unit shall be a prerequisite for the six- to twelve-month schedule given in Section B.

Insofar as possible, the majority of the individual's time in both A and B should be spent at the bedside or in the clinical laboratory. The one-week survey courses will be planned primarily for the practicing physician and will specifically exclude operative techniques. Clinics, demonstrations, ward rounds, conferences, and round table discussions will suitably compose a major part of these courses, whether long or short. Reading of pertinent literature will be suggested. Individuals taking the longer courses may, in part, be assigned to ward services as junior attending physicians, depending, of course, upon their respective qualifications. Visual education will be of the utmost value in supplementing all of these functions, and, in fact, a great service will be done to medical education generally by the foundation or Governmental agency which subsidizes a broad program of assistance to medical schools in preparing their own colored movies and lantern slides of various aspects of disease.

Finally, it would be well if a national clearing house or central committee were to be established now which would at once begin to prepare a list of all available locations for graduate and postgraduate education in American medicine. Quite possibly some committee already existing within the structure

*In conjunction with Dr. W. A. O'Brien, Director of Postgraduate Medical Education, University of Minnesota.

of a representative organization will undertake this task.* Such a committee could advise candidates as to the minimum period of training that they should receive on the basis of their prewar and war experience either in general medicine or in some special field. At the same time, an applicant could be informed that a fellowship, house officership, or refresher course, as best fitted for his particular case, might be applied for at a given institution. Centralization along these general lines should be of material aid in providing the largest possible number of places and in accomplishing this end in the best interests of medical care and clinical research.

*Since this address was given, a letter has been received from the Council on Medical Education and Hospitals, and the Committee on Postwar Medical Service, of the American Medical Association, indicating that they will act in this capacity.

Scientific Program

CHANGES IN THE PRECORDIAL ELECTROCARDIOGRAM PRODUCED BY EXTENSION OF ANTEROSEPTAL MYOCARDIAL INFARCTION

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An electrocardiographic study of two patients, each of whom experienced two attacks of severe chest pain of the anginal type within a short period of time, illustrates the value of multiple precordial leads in diagnosis of the extension of an anteroseptal infarct.

In the first case, the limb leads taken six and one-half hours after the onset of symptoms are negative except for slight terminal inversion of the T waves in Leads I and V_L, changes somewhat suggestive but not diagnostic of recent myocardial infarction. The multiple precordial leads, however, show QRS and T-wave changes in V_2 and sharply inverted T waves in Leads V_3, V_4, and V_5. Approximately thirty-six hours after the first attack of pain, the patient had a second more severe and prolonged period of substernal distress associated with marked prostration. This attack was accompanied by the appearance of characteristic and progressive QRS and T-wave changes in Leads I, V_L, V_3, V_4, and V_5, indicating a lateral extension of the infarcted zone of muscle.

The second patient was first seen approximately eighteen hours after several sharp attacks of atypical angina pectoris. The electrocardiograms taken at that time display prominent QS deflections and inverted T waves characteristic of myocardial infarction in Leads V_1, V_2, and V_E. Sharp terminal inversion of the T waves is seen also in Leads I, II, V_L, V_3, V_4, V_5, and V_6, but no significant changes in the QRS complexes appear in these leads. Two days after the examination, the patient experienced a prolonged attack of severe chest pain. The electrocardiograms taken three months later show QS or large Q waves and sharply inverted T waves in Leads V_2, V_3, V_4, and V_5, indicating that the zone of infarction had extended laterally.

The observations made illustrate the worth of multiple unipolar precordial leads in the diagnosis of the extension of an anteroseptal infarct. They also suggest that, in patients with coronary arterial disease, at least some of the attacks of pain which are usually considered premonitory or prodromal symptoms of myocardial infarction represent the development of a small infarct and that the more characteristic symptoms of coronary thrombosis which often occur later are due to an extension of the initial lesion. Evidence is again presented that infarcts which are anteroseptal in location, as shown by diagnostic changes in leads from the right precordial area, often fail to produce equally significant changes in the limb leads.

DISCUSSION

DR. L. N. KATZ, Chicago, Ill.—The evidence presented by the Ann Arbor group would be more convincing if they had obtained control electrocardiograms preceding the recent myocardial infarction. The possibility remains that the patient might have had an old healed infarct to account for the appearance of the first record. There are cases on record in which following severe precordial pain, the electrocardiogram, including chest leads, has been normal or not characteristic. While the results of the authors are suggestive, their argument would be greatly strengthened if the possibility that the first record shown was not due to an old, healed infarct had been established.

DR. DOUGLAS DEEDS, Denver, Colo.—I was interested to note from Doctor Rosenbaum's slides that the central terminal lead from the left arm exhibited inversion of the T wave while the same lead from the right arm and left leg did not. Two years ago it was my privilege to present to this Society electrocardiographic data based upon the comparison of precordial Leads CR, CL, CF, and CT in an increasing series of abnormal cases now numbering in the hundreds. For several years it has been my belief that the best routine precordial lead technique consists of Leads CL_2, IV_L, CL_6, and the Whitten amplification of Lead I. Therefore, I was keenly interested in noting the corroborative evidence supplied by Doctor Rosenbaum's slides in which the central terminal lead from the left arm exhibited T-wave inversion, whereas the same lead from the right arm and left leg exhibited upright T waves. Continuing investigation continues to demonstrate that not infrequently the central terminal technique nullifies the valuable "sensitivity" of the Cl leads in acute cor pulmonale and in posterior left ventricular infarction and strengthens my belief that the left arm is the best so-called indifferent electrode to use in routine precordial lead electrocardiography.

DR. ROSENBAUM (closing).—I agree with Doctor Katz' suggestion, that if earlier, control electrocardiograms had been available, the observations might be somewhat more impressive. It should be noted, however, that the first patient had no cardiac symptoms whatever prior to those which appeared six and one-half hours before the first standard leads and twenty-seven hours before the precordial leads were taken. This additional slide presents the records of a patient who had a myocardial infarct two or three weeks earlier. Except for slight flattening of the T waves in Lead I, the standard leads and the leads from the left precordium (V_4, V_5, and V_6) are well within normal limits. However, the unipolar lead from the left arm (V_L) shows sharp terminal inversion of the T waves and V_2 and V_3 (from the right precordium) display QS deflections and inverted T waves characteristic of recent anteroseptal myocardial infarction. Some of the cases of "impending myocardial infarction" which have been reported to show no or minor changes in the electrocardiograms, which have been limited to the standard leads and a single lead from the left precordium (usually IVF), may actually have been similar examples of anteroseptal myocardial infarction. Again, I would like to emphasize that we do not feel there is sufficient evidence at the present time to indicate that all premonitory symptoms are associated with infarction. There are probably many instances in which these manifestations are not associated with actual infarction.

In reply to Doctor Deeds' question regarding the T waves in the lead from the left arm (V_L), it is true that in both of these patients the T waves were inverted in this lead. This is the usual finding in infarcts which involve the anterior aspects of the left ventricle. However, if the left arm were used as the indifferent point in taking the precordial leads, the effect would be to subtract the inverted T wave of the unipolar left arm lead from the negative T wave of the precordial leads, thereby diminishing rather than increasing the evidence of infarction.

THE USE OF THE AUGMENTED UNIPOLAR LEFT LEG LEAD IN THE DIFFERENTIATION OF THE NORMAL FROM ABNORMAL Q WAVE IN STANDARD LEAD III

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An attempt was made to evaluate the diagnostic significance of the QRS pattern in the augmented unipolar left leg lead (Lead AV_F) as a means of establishing or excluding the diagnosis of posterior infarction. A series of forty-nine patients was selected for study because of the presence of a prominent Q wave in standard Lead III. One additional patient with posterior infarction was included who did not have Q waves but exhibited the classical ST-T wave changes in Lead III.

Multiple precordial and unipolar extremity leads were taken on every patient and esophageal leads were taken on forty-four of the fifty patients. The presence of a posterior infarct was established in a total of twenty-five patients—in four of these by autopsy and in the remaining twenty-one by typical esophageal leads. The infarct was months or years old in all but four patients. Posterior infarction was excluded in a total of twenty-five patients—in three by autopsy and in the remaining twenty-two by negative esophageal leads.

In all cases where posterior infarction was excluded, a prominent Q3 or QS3 was present. This amounted to 25 per cent or more of the tallest R in twenty-three of the twenty-five patients; from an examination of the standard leads alone, many of these cases could not be distinguished from cases proved to have old posterior infarction. The pattern of the QRS in lead AV_F proved to be of considerable help in this differentiation.

A QAV_F which was 25 per cent or more of RAV_F was found in twenty-two of the twenty-five patients proved to have posterior infarct, and in only three of the twenty-five patients in whom the diagnosis of posterior infarction had been excluded. In both patients with posterior infarct who had a Q3 but failed to show QAV_F, esophageal leads suggested that the infarct was located high on the posterior wall, near the auricular margin. The voltage of the QRS wave in Lead AV_F was low in two of the three uninfarcted controls who showed a Q/R ratio exceeding 25 per cent in this lead. In the remaining case, the deep Q wave was present in Lead AV_F when the patient was recumbent, but disappeared when the curve was taken in the erect posture.

DISCUSSION

DR. EDWARD MASSIE, St. Louis, Mo.—In the Heart Station at Barnes Hospital, we routinely have the patient take a deep breath in Lead III. It has been our impression that, in those patients with normal hearts, the Q3 tends to disappear or at least become significantly smaller following a deep inspiration. I should like to ask for other opinions regarding this point, because certainly this would be a simple way to differentiate a normal from an abnormal Q3.

DR. J. S. LADUE, New Orleans, La.—I would like to ask whether or not any correlation between the anatomic axis of the heart was made in patients who had prominent Q waves in the augmented unipolar leg lead but who failed to show infarction at autopsy. The horizontal position of the heart might account for the enlarged Q.

Dr. Louis N. Katz, Chicago, Ill.—The term unipolar lead is misinforming. It still remains to be demonstrated that these leads show only the potential changes of one extremity, and I wish it to go on record that I believe this concept is far from proved.

Dr. Franklin D. Johnston, Ann Arbor, Mich.—I think the chest lead is worth while. I would like to point out one further point, however—the importance of potential variation of the left leg in posterior infarction. The left leg, of course, enters into the production of II and III standard leads. Furthermore, the fallacy of the left leg in these two leads is the same. Therefore, if alterations in the potential of the left leg are going to produce a Q wave in Lead III, they will also produce Q waves in Lead II. That particular point of the appearance of Q waves in Lead II as well as in Lead III is of significance. Several years ago Durant made a study of a small group of patients in a heart station and pointed out the importance of having small Q waves in Lead II as well as Q waves in Lead III.

Dr. Chester M. Kurtz, Madison, Wis.—For several years at the Wisconsin General Hospital we routinely have been taking a strip of Lead III with the breath held in deep inspiration and have found it a very valuable procedure. In general, a prominent Q3 resulting from myocardial infarction or serious coronary sclerosis tends to persist, while a Q3 which is of no clinical significance is very likely to disappear. I do not mean to imply that this procedure can in any way replace the method outlined by Doctor Myers but simply mention it in corroboration of the experience obtained by the group at Barnes Hospital in St. Louis.

Dr. Myers (closing).—Both Doctor Massie and Doctor Kurtz have called attention to the well-known tendency of deep inspiration to obliterate the normal Q3. This did not take place in some of our patients having a normal Q wave in Lead III. Furthermore, respiratory changes in amplitude may occur in the Q3 associated with posterior infarction. Hence, the practice of taking a portion of Lead III during deep inspiration is a helpful but not absolutely conclusive procedure in the differentiation of the normal from the abnormal Q3.

With reference to Doctor LaDue's comment, we are attempting to make a correlation of the electrical axis observed during life with the anatomic axis observed at post mortem. There seems to be a rough correlation but the study has not progressed far enough to justify any positive statement.

Doctor Katz is technically correct in objecting to the use of the term "unipolar" since the potential of the "indifferent" electrode in the Goldberger as well as in the Wilson terminal is not exactly zero. The potential of the "indifferent" electrode, however, is so small it does not detract from the clinical use of Lead AV_F in the diagnosis of posterior infarction.

Doctor Johnston has brought out the fact that in posterior infarction an abnormal Q wave is often present in Lead II as well as in Lead III. This is certainly true in the acute stage, but as time passes the Q wave tends to disappear from Lead II while it remains in Lead III. In twelve of our twenty-five patients with posterior infarct, Q2 was either absent or less than 25 per cent of the amplitude of R2.

OBSERVATIONS ON THE HUMAN ELECTROCARDIOGRAM DURING EXPERIMENTAL DISTENTION OF THE GALL BLADDER

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(INTRODUCED BY N. C. GILBERT, M.D.)
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The association of organic heart disease and biliary tract disease, especially stone-bearing gall bladders, has been emphasized by clinicians for many years. Physiologists have demonstrated the presence of viscerocardiac reflexes, especially in the lower animals, and more recently experimental work on animals has demonstrated reduced coronary blood flow on distention of the stomach and gall bladder. With these ideas in mind, we felt that the study of the distended human gall bladder with the electrocardiographic method might yield further evidence indicating the close relationship between the heart and the gall bladder. It is obvious that the results of such a study are of much importance to the surgeon and internist.

Procedure.—In the present series of seven female patients whose ages ranged between 34 and 78 years, all had stone-bearing gall bladders and cholecystectomy was performed on each patient. In the preoperative clinical study, electrocardiograms were made as controls. The preoperative medication usually consisted of $\frac{1}{6}$ to $\frac{1}{4}$ gr. of morphine sulfate hypodermically and $\frac{1}{100}$ gr. of atrophine sulfate or $\frac{1}{150}$ gr. scopolamine in some cases.

Control electrocardiograms were made with a portable instrument while the patient was still awake on the operating table. Additional tracings were made as the patient was going under and especially during the third plane of anesthesia. Cyclopropane was the usual anesthetic; in some instances a mixture of cyclopropane and ethylene was used. During crucial procedures most observations were made with Lead II. Precordial leads were omitted entirely, because the chest lead might be in the surgeon's way and, what is probably more important, we wished to approach the subject with a more stable lead, so Lead II was our choice. When there was time, some observations were also made with Leads I and III.

After the gall bladder was isolated, about 30 c.c. of normal salt solution were injected suddenly through a fine hypodermic needle into the fundus of the gall bladder. In some cases quite a high degree of pressure was obtained in the gall bladder, especially if the cystic duct was blocked with a stone. Tracings on Lead II were also made of such procedures as cutting the skin, pulling the peritoneum, and traction on the gall bladder and cystic duct. Tracings were made during convalescence.

Summary.—

1. During the experimental distention of the human gall bladder, we have observed either an increase in heart rate (four patients) or no change in rate at all (three patients). In our small series, slowing of the heart rate has not been observed. When the heart rate is going to accelerate after distention of the gall bladder, the increase in rate usually occurs immediately. After release of the pressure in the gall bladder, the slowing down of the heart rate is a gradual affair.

2. Depression of T II to the isoelectric level has been observed; complete negativity of T II has not been seen so far.

3. Extrasystoles occur frequently after distention of the gall bladder.

4. Increased P-R interval (partial block) has been observed. The block disappears rather quickly after release of the pressure in the gall bladder (by aspiration of contents).

5. Traction on the gall bladder or cystic duct in some patients speeds up the heart rate almost immediately.

6. In our experience, the heart rate usually slows down during the third plane of anesthesia, before surgery is started, either with cyclopropane or a mixture of cyclopropane and ethylene. There is also a better definition of all the components in the electrocardiogram. We have seen little arrhythmia or conduction disturbances during the third plane of anesthesia in our control tracings.

7. Cutting the skin and dissection of the subcutaneous tissues down to the peritoneum usually produced no changes in the electrocardiogram. In one patient, pulling on the peritoneum speeded up the heart rate.

Conclusion.—These observations indicate that it is impossible to predict what will happen in the human electrocardiogram after distention of the gall bladder. There is no specific reaction or pattern in the electrocardiogram which is characteristic of gall bladder distention. In some patients distinct changes occur immediately in the electrocardiogram after distention of the gall bladder with normal salt solution; in others, little or no reaction occurs.

These observations are to be regarded as a preliminary report. Further observations with some modification of technique are being made. The studies will be reported subsequently after a much larger series has been observed. We have been unable to find any similar published reports in the literature in which the human gall bladder has been distended with normal salt solution and studied with the electrocardiographic method.

DISCUSSION

Dr. Joseph A. Capps, Chicago, Ill.—These findings are quite interesting. The interpretation of the mechanism by which dilatation of the gall bladder produces heart block is rather puzzling. We might consider as an analogy the effect of traumatizing the inflamed visceral pleura, since the pleura has the same innervation as the gall bladder.

Prolonged irritation of the inflamed visceral pleura often produces slowing of the pulse, and at times actual stoppage of the heart. This is a cardio-inhibiting reflex. So the heart block resulting from dilatation of the gall bladder would suggest a vagal effect.

Commonly enough the pulse rate in the essayists' experiments was not slowed. It may be that the atropine administered preoperatively counteracted the slowing effect of the vagus nerve but did not prevent impulses from passing to the bundles of His, resulting in transient heart block. I would suggest to the authors that further observations be made with the omission of the atropine.

Dr. Donald D. Kozoll, Chicago, Ill.—I would like to ask the investigators if they attempted distention of the common bile duct with these observations.

Dr. G. K. Fenn, Chicago, Ill.—I was interested because we reported the results on the coronary circulation with distention of the hollow viscera some

time ago. I would like to ask if the authors noticed any clinical evidence of effect on the coronary circulation.

DR. WAKEFIELD (closing).—I think that Doctor Capps' point about atropine is a good one. I do not know the answer in fact. I certainly would say that the dose that is used was not great enough, 1/100 to 1/150 gr., to paralyze the vagi. Otherwise we would not get any response because the work of Fenn and Gilbert has shown where the vagi are paralyzed in dogs, either by section or paralysis, you do not get any reaction.

Regarding Doctor Kozoll's question about distention of the common duct itself, we have not done that in the operating room. Our immediate problem was the effect of the distention of the gall bladder itself. We distended the common duct in a patient postoperatively in whom Doctor McArthur had left a T tube. However, that is nothing new because Ravdin has made a demonstration of what happens there. We found the same result. We did not have a ballisto-cardiograph and we could not determine the cardiac output as Starr has done, but we did have the patient connected to the electrocardiograph and distended the common duct through the T tube to the point where it produced substernal pain and there was no change in the pulse rate and very little fluctuation in blood pressure and no change in the tracing. I think Ravdin in 1942 before the American Surgical Association spoke of the same thing. We considered that another problem. The results have not been as interesting as what happened in the distention of the gall bladder itself.

Doctor Fenn's question is interesting as to what clinical results we have seen in these patients by getting rid of a stone-bearing gall bladder in which there may have been an anginal syndrome. There have been several dramatic results by removing a stone-bearing gall bladder. The woman in whom we produced the complete heart block by traction on the gall bladder had had angina pectoris for years. One year before surgery she had an anterior infarction and she had this stone-bearing gall bladder. She could hardly walk a block without using five or six nitroglycerine tablets. Her immediate remark postoperatively was that her chest felt better than it had in years. I understand she can walk a block at ordinary pace without any pain. I do not think gall bladder surgery is going to cure all of the patients entirely. I think if the patient referred to should walk fast enough with a full stomach and against a cold head wind she will get the pain. The pain is minimized and she feels better. She is not having any more gall bladder colics.

GENESIS OF CRUSH SYNDROME*

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A state which resembles that clinically described as crush syndrome may be produced in rats by intravenous injection of metamyoglobin after release of compression from one crushed hindlimb. This renal injury is apparently of dual origin, arising (a) in renal vasoconstrictive ischemia, which follows skeletal trauma (Corcoran, Taylor, and Page, Ann. Surg., 1943), and (b) in intratubular, possibly intracellular, deposition of metamyoglobin and release of hematin.

The means of treatment suggested by this concept of the genesis of crush syndrome were explored. Intravenous injection of sodium bicarbonate or citrate, elsewhere recommended, was found to be contraindicated. Varying degrees of

*This work was done under contract with the Committee on Medical Research, Office of Scientific Research and Development.

protection were obtained by (a) injection of diuretics—such as sucrose, mannitol, or sodium sulfate, (b) application of a compression bandage to the limb after release of crush, and (c) injection of a colloidal plasma substitute, such as gelatin alone, or, better, in M/6 sodium lactate. The most satisfying results, as shown in the prevention of mortality and of renal functional and structural impairment, followed use of a combined treatment in which a pressure bandage was applied to the crushed limb and gelatin in sodium lactate injected intravenously.

DISCUSSION

DR. P. K. KNOEFEL, Louisville, Ky.—The application of a tourniquet would lead to reduction in circulating blood volume which would undoubtedly reduce the local blood flow per se and cause vasoconstriction. That might serve as an explanation without introducing any circulatory substance. I would like to ask for an explanation of how that could be ruled out.

QUESTION.—What do you mean by compression bandage?

DR. CORCORAN (closing).—The compression bandage we used was designed by Doctors Taylor and Page. It is of an elastic weave and is permeated with an extensible plastic. It was applied spirally from toes to groin after release of the crushing cords and left in place thereafter.

The basis for its use lies in the fact that the renal ischemia elicitable by partially occluding limb tourniquets is almost entirely prevented by prior application of snug plaster encasements. This ischemia is only very incompletely relieved or prevented by renal denervation or by transfusion of large volumes of blood. Its onset is associated with the appearance in plasma of a substance which causes vasoconstriction of the isolated rabbit's ear. It seems likely that this vasoconstrictor is the major cause of renal ischemia in skeletal trauma. Its release from the site of injury is inhibited by snug plaster encasements: thus, the compression bandage used was intended to prevent renal ischemia and the gelatin-lactate solution to restore plasma and interstitial fluid volume and initiate diuresis. The two etiologic factors of crush syndrome were thus simultaneously attacked.

EVALUATION OF PECTIN AND GELATIN SOLUTIONS USED IN THE TREATMENT OF SHOCK: HISTOLOGIC CHANGES PRODUCED IN THE HUMAN BEING

HANS POPPER, M.D.* (BY INVITATION), BRUNO W. VOLK, M.D.* (BY INVITATION), KARL A. MEYER, M.D. (BY INVITATION), DONALD D. KOZOLL, M.D. (BY INVITATION), AND FREDERICK W. STEIGMANN, M.D.*
CHICAGO, ILL.

A comparison of the effects of 257 liters of gelatin given to 162 patients and of 235 liters of pectin given to 155 patients was made from a therapeutic, hemodilution, hematologic, and histologic point of view. Approximately half of the patients were in shock and the others were hospital controls.

Previous work has shown an almost identical effectiveness of similar quantities of both these solutions in the treatment of patients with shock produced by a variety of causes despite a marked difference in chemical nature, concen-

*At present in service with the Armed Forces.

tration, and physical characteristics. Furthermore, both produced a comparable hemodilution in regard to degree, duration, and a relative leveling off of hemodilution with larger doses. This leveling off (or decreasing hemodilution with increasing amounts of the solution) is more marked in patients with anemia or hypoproteinemia. The hypothesis is suggested that the hemodilution is started by the macromolecular solution but maintained by other substances, possibly mobilizable tissue proteins.

The increase of the sedimentation rate was selected as an example of the hematologic changes. It was of similar degree with both pectin and gelatin and showed the same tendency to level off with higher doses.

The two substances differed decisively in the production of tissue changes as observed from the necropsies of twelve patients given gelatin and ten patients given pectin at varying intervals prior to death and from the liver biopsies of eight patients, four of whom were given pectin and four gelatin prior to operation. Gelatin produced only a transient vacuolization of the proximal convoluted tubules of the kidney similar to that seen after sucrose. In four of ten patients who received rather large amounts of pectin, splenomegaly was produced. Histologically, the deposition of a peculiar material in the reticulo-endothelial cells of the spleen, Kupfer cells of the liver, glomerular tufts of the kidney, and alveolar cells of the lung was prominent. Vacuolization of the epithelium, endothelial cell proliferation, giant cell formation, and hyalinization of renal glomeruli were seen. Although it is difficult to identify this material chemically, it does stain with ruthenium red, a dye used for the staining of pectin. It resembles amyloid in deposition but not in staining reaction. The same picture was reproduced in five rabbits given varying amounts of pectin; rabbits receiving gelatin showed insignificant changes. These findings indicate that pectin is a reticulo-endothelial irritant while gelatin does not produce tissue reaction.

DISCUSSION

Dr. Charley J. Smyth, Eloise, Mich.—I think that Doctor Popper's presentation is very important because it shows the limitations and the potential dangers of pectin administered intravenously. About two years ago at the Eloise Hospital, Detroit, Mich., we undertook a study to evaluate the various substances advocated for use in the treatment of patients in shock. Through the courtesy of Doctor Frank Hartman, of the Henry Ford Hospital, we obtained some pectin which was used to study its influence on the plasma volume of normal human subjects. In these investigations Evans' blue dye No. 1824 was used. In each of five patients in whom we injected pectin and the dye a generalized purpuric eruption occurred approximately nine days following the injection. In similar studies using 5 per cent gelatin (Upjohn) in which both gelatin and the dye were used we observed no untoward effects. In addition, we have treated approximately 150 patients with 5 per cent gelatin in an attempt to evaluate its effect in controlling shock in patients undergoing surgical operations, and in none of them have we seen any deleterious reactions which we could attribute to gelatin.

Dr. Kozoll (closing).—I can only confirm what Doctor Smyth said about the eruption. In three patients who received 5,000 c.c. or more of pectin in divided doses, we saw this hemorrhagic macular rash over the extensor surface

of the extremities and no dye was administered. It appeared to us that possibly this occurrence with Evans' dye was coincidental. The patients did not present any hematologic changes and the rash was of no significance.

CHANGES IN THE TREATMENT OF TOXIC GOITER PRODUCED BY THIOURACIL

W. O. Thompson, M.D., and P. K. Thompson, M.D. (By Invitation)
Chicago, Ill.

Most of what has been written on the treatment of toxic goiter up to the present time is out of date. Data thus far available appear to warrant the following conclusions:

1. In most patients with toxic goiter, thiouracil, when administered in proper doses, will cause a reduction in basal metabolism to within normal limits and maintain it there indefinitely.

2. In some patients, after the basal metabolism has been maintained at the normal level for six months or more by the administration of thiouracil the drug may be omitted without any increase in basal metabolism occurring.

3. It, therefore, appears probable that in patients who are not hypersensitive to the drug and who do not develop serious toxic reactions, toxic goiter may be treated by the administration of thiouracil alone. It will, of course, take several more years to determine the precise status of the drug with regard to the actual percentage of cures and the presence or absence of untoward late complications.

4. When surgical measures are employed, the drug is of great value in preoperative preparation, particularly in patients who have the disease in a very severe form and cannot be prepared by the various measures commonly employed before the introduction of thiouracil. In nearly all such patients who are not hypersensitive to the drug long periods of hospitalization before operation may be avoided by its administration.

UNUSUAL REACTIONS FOLLOWING THE USE OF THIOURACIL

Samuel F. Haines, M.D., and F. Raymond Keating, Jr., M.D. (By Invitation)
Rochester, Minn.

This report deals with two patients with severe recurrent exophthalmic goiter who were treated with thiouracil. In both instances the thyroid gland was not palpably enlarged but symptoms of hyperthyroidism were severe and the basal metabolic rate was greatly elevated. In each case the administration of thiouracil was followed after several days by diminution of the signs and symptoms of hyperthyroidism and by lowering of the basal metabolic rate. Transient painful swellings of subcutaneous tissues occurred; at the same time, the value for the plasma chloride was increased and the carbon dioxide combining power of the plasma was decreased. In one patient, myoclonic contractions of various muscles, more particularly of the facial muscles, occurred, and at the same time severe somnolence and confusion were present. The myoclonic twitchings persisted for several days after the administration of thiouracil was stopped. Sub-

sequent administration of the drug on two separate occasions was followed rapidly by a resumption of the untoward symptoms and signs. In the other patient, a similar state of somnolence and mental confusion occurred during a second period of administration of the drug. It is felt that these reactions probably were dependent upon a toxic disturbance of the central nervous system.

Since the presentation of this paper, a reaction similar to the foregoing has been produced in one of these patients by the administration of an inert substance. This raises a question as to the exact cause of the previous reactions in both patients. Further study in an attempt to determine more of the nature of the reactions is being carried out.

DISCUSSION

DR. M. A. BLANKENHORN, Cincinnati, Ohio.—I would like to ask Doctor Thompson to elaborate a little more on the management of the patients in addition to the drug therapy. In the older days when surgical and medical methods were compared, it was often shown that the circumstances under which the patient had to live during the period of medical management had a psychologically bad effect while that with surgery was psychologically good. Would Doctor Thompson describe the other circumstances?

DR. C. C. STURGIS, Ann Arbor, Mich.—This treatment has been instituted in all patients with toxic goiter on the medical wards of the University of Michigan Hospital. To date, about thirty patients have been observed and followed long enough to judge the immediate results. Without exception all have had a decrease in the basal metabolic rate and other gratifying evidences of improvement. My experience, therefore, agrees that its therapeutic effects are most promising.

Three of our patients had a leucopenia and an additional one a febrile reaction. Associated symptoms, when present, were mild, and in all instances the drug was resumed without incident after it had been discontinued for a few days. Tentatively, we believe that untoward symptoms are most likely to occur soon after the treatment is begun, but they may appear after the therapy has been given for a long time.

Some surgeons complain that following thiouracil therapy the thyroid gland develops increased vascularity which causes excessive bleeding if the patient is operated upon. This may be averted, however, if iodine is also given for two weeks prior to the operation. Occasionally, malignancy may develop in a patient with a toxic adenoma during the course of treatment, as it did in one of our patients.

DR. MARTIN GOLDNER, Chicago, Ill.—My observations at the University of Chicago are based on a series of about twelve patients. Those were patients that had refused operation and had undergone a relapse. All those had proved to be iodine-resistant. On the basis of these observations we can confirm Doctor Thompson's observations on the effect of the drug. In our uncomplicated cases we found that one month, two months, or three months of treatment is necessary. Also we have seen a high percentage of toxic complications—I would say in about 30 per cent of our patients, which is quite a number in a small group. Three or four showed toxic symptoms. There was a severe toxic psychosis in one patient. After treatment of about one month in the dosage recommended, there were no organic findings and no changes in the blood count. The second patient was regarded as already toxic with a generalized adenopathy. This patient was treated with the same dose for three weeks and developed fever and generalized swelling of the lymph glands around the neck. The drug was discontinued. In three days the high temperature decreased and the swelling of the glands disappeared. After one week the same dose was repeated and

the same symptoms occurred. An interesting observation was that patients who proved to be iodine-resistant after thiouracil treatment responded to iodine.

DR. G. T. EVANS, Minneapolis, Minn.—I would like to report on a series of twenty-six cases at the University of Minnesota Hospitals. For the most part, the results have been very gratifying. However, five toxic reactions were observed. One was a severe rash. In another patient with marked leucopenia we saw the neutrophiles go from over 1,000 to under 100 in a two-day period; although the drug was stopped, this lasted into the fourth day thereafter. Unless counts are made frequently, reactions like this will not be caught in time. A toxic manifestation which has not received much attention is spontaneous purpura. This occurred in one patient. In two subsequent patients the cuff test was routinely done and became markedly positive under thiouracil therapy. We believe that observations of bleeding tendency will need attention in the administration of this drug.

DR. PAUL H. NOTH, Detroit, Mich.—We noticed severe cerebral toxic manifestations followed by exitus apparently due to thiourea in a Negro man 40 years of age who suffered from a nodular goiter with associated thyrotoxic heart disease. The cardiac failure was controlled by rest and digitalis. Variable glycosuria and fasting blood sugar levels ranging between 118 and 148 mg./100 c.c. were attributed tentatively to the hyperthyroidism. On the twenty-fifth day following admission thiourea was administered orally in a dose of 1 Gm. every six hours. Five days later the patient appeared weak and complained of dizziness. The next day he vomited on one occasion. That evening he became irrational, restless, semicomatose, and incontinent. Respirations were shallow and rapid. The thiourea was discontinued. The level of blood sugar increased to 396 mg./100 c.c. and the blood urea to 79 mg./100 c.c., but the CO_2 combining power showed only a moderate reduction to 36 vol. per cent. Leucocytes of the blood numbered 13,800. The lack of clinical and laboratory evidences of diabetic acidosis excluded this as the cause of his symptoms. Therapy consisted of the usual measures for control of diabetes. The blood sugar level fell slightly and the CO_2 combining power remained at about the same level. The patient's mental state did not change and he died two days later.

Another patient with hyperthyroidism and diabetes showed an otherwise unexplained rise of blood sugar following administration of thiourea.

DR. W. H. BUNN, Youngstown, Ohio.—We have twenty patients who have taken thiouracil. Our experience has been very much the same as those cited. We have had one patient who developed severe jaundice. The pharmaceutical house that sent us our supply of thiouracil told us they had had other cases of jaundice reported.

Our plan has been to stop iodine while thiouracil is being given because it seems to slow its effect. For the surgeon's benefit we give iodine for ten days before operation in fairly large doses. The gland "firms" up and there is no trouble from excessive bleeding.

DR. W. O. THOMPSON, Chicago, Ill.—So far as the psychologic factors in the production of toxic goiter are concerned, it seems to me these are not very important in most instances and have been greatly overdone. Certainly the disease is not psychogenic in origin. There may be occasional instances in which some unusual psychic trauma seems to set off the disease. By and large, however, it is a chemical disturbance. Psychiatrists have developed a great variety of peculiar explanations for many diseased states. After reading some of these explanations one is almost forced to the conclusion that they belong in the realm of stargazing. As our knowledge increases we must conclude that most diseased states can be explained by organic or chemical alterations in the body. Please do not misunderstand me; I am not claiming that all psychiatrists are star-

gazers, but in the past there has been some tendency in this direction. I am very happy to see that one of the most important recent advances in the field of psychiatry is an effort to correlate mental and emotional changes with organic and chemical changes in the central and sympathetic nervous systems.

The pulse rate follows the basal metabolism and actually shows the same change that it does following a reduction in metabolism from any other cause. So far as we can tell, when the metabolism has been reduced by thiouracil administration, the clinical condition of the patient is exactly the same as when the basal metabolism has been restored to normal by removal of the gland.

One word about toxic reactions. This, of course, is a drug not to be used indiscriminately; it is still in the experimental stage, and it is still not available commercially. It will take two or three years more to determine its precise status. Some patients have a definite sensitivity to the drug. One woman, following a single dose of 0.1 Gm., showed an increase in temperature to 103° F. with pain in the legs, diarrhea, nausea, and vomiting. That phenomenon was repeated twice. If we had persisted in the administration of the drug to that patient, the result might have been fatal. It is wise to give an initial dose of 0.1 Gm. to see if there is any sensitivity. The patient afterward must be followed with great care. White counts must be taken at frequent intervals. In spite of all these toxic reactions, it seems to me that the introduction of thiouracil represents a very great step forward and provides us with an important clue as to what is going on in toxic goiter.

Dr. Keating (closing).—We have been impressed with the fact that toxic reactions may take place at any time. We have observed them as early as twelve days after treatment with thiouracil has been begun and encountered a severe toxic rash and fever in one patient who had been taking thiouracil for five and one-half months. The fact that several months have passed does not permit the doctor to relax his caution.

A point which has not been emphasized is the possibility of postoperative crises in patients inadequately prepared for surgery with thiouracil. We have not encountered any, but such reactions have been mentioned by some workers. We feel there is a serious risk in assuming that thiouracil and iodine act identically in the preparation of patients for surgery. Patients with exophthalmic goiter who have been given iodine may sometimes be operated on with comparative safety while the basal metabolic rate is still elevated. Until more is known about it, I feel that it is hazardous to assume that this is the case in patients prepared for surgery with thiouracil.

THE UPPER LIMITS OF HEAT AND HUMIDITY TOLERATED BY ACCLIMATIZED MEN WORKING IN HOT ENVIRONMENTS*

Major William B. Bean, Major Ludwig W. Eichna (By Invitation), and Major William F. Ashe (By Invitation)
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Defining the environmental ceiling for work in the heat is a complex problem. One must control state of acclimatization, nature of environment, work, fitness, and morale of the subjects, their clothing, diet, and salt and water intake. The condition of the men during and after work and its "cost" to the subject in terms of physiologic disturbances indicate the upper limits. Because of many possible combinations of heat, humidity, wind, radiation, work, and clothing, an arbitrary set of conditions was chosen for investigation. Thirteen healthy young garrison troops were first acclimatized to dry and moist heat. They then

*From the Armored Medical Research Laboratory, Fort Knox, Ky.

worked in fourteen environments approaching and exceeding tolerable environmental limits for work. The environments varied only in dry and wet bulb temperatures; no radiant energy was supplied. Air movement was that created by the unclothed men walking single file at three miles per hour around the laboratory hot room on a seventy-seven foot track, carrying a twenty-pound pack. The work consisted of walking continuously for four hours. The energy expenditure was approximately 300 calories per hour. Water salted to 0.1 per cent was given when requested and replacement of that lost as sweat was urged. Data were obtained for various combinations of D.B.T.* between 93 and 121° F. and W.B.T.† 90 and 96° F. and the conclusions apply only to this range of environments.

The W.B.T. is the important limiting factor determining the ability of men to work in hot environments. The D.B.T has a minor influence. The limiting W.B.T. is approximately 2° F. higher at a D.B.T. of 100° F. than at 120° F.

Near the upper limits, the range of W.B.T. from an environment in which men work with "relative ease" to one in which work is "impossible," is very narrow: 4 to 5° F. for a given D.B.T.

Below a W.B.T. of 91° F. men work easily and efficiently.

Between W.B.T. of 91° and 94° F. men can work but with difficulty, loss of vigor and alertness, and undesirable physiologic changes.

At W.B.T. of 94° F. and higher, less than an hour of sustained work is possible; acute disability with marked physiologic changes occurs.

Near the upper limits sweating averaged 2.5 liters per hour and reached 3.5 liters in some men.

Undesirable physiologic changes encountered near the upper limits include: (1) Heart rates of 150 to 180; (2) rectal temperatures of 102 to 103.5° F.; (3) average (weighted) skin temperatures approaching 101° F.; and (4) violent and protracted headache, nausea, copious vomiting, abdominal cramps, severe weakness, vertigo, dyspnea, paresthesias, stumbling, disorientation, and collapse with syncope.

METABOLIC ACCLIMATIZATION TO TROPICAL HEAT

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With the large military forces now seeing enforced activity in regions of tropical moist heat, it is especially important that most careful consideration be given to the effects such heat may exert. Unfortunately, several recent articles from investigators engaged in government-sponsored research projects have presented an incomplete and erroneous picture of what may be expected in such acclimatization. While *vasomotor* adaptation to hot environments is usually complete after three to five days of exposure, all studies have tended to indicate that *metabolic* acclimatization takes place much more slowly—that it begins late in the second week of continuous exposure and is largely accomplished by the end of the third week.

*D.B.T., Dry bulb temperature.
†W.B.T., Wet bulb temperature.

Previously published and new evidence will be presented to prove the existence of this two- to three-week lag in metabolic adaptation and to deal particularly with the question of heightened thiamine and choline needs in tropical heat. New studies have shown that the heightened need for these two factors continues even when the diets contain 0.5 per cent sulfaguanidine; hence, heat depression of intestinal synthesis cannot account for the findings.

The heightened need in tropical heat is most emphatic for choline. Animals on choline-free diets in the heat and cold show no difference during the first week, and during the first half of the second week acute hemorrhagic nephritis causes an equal number of deaths in the two environments. After the second week, however, growth in the cold goes on practically as well without as with choline in the diet, while in the heat, growth is sharply affected.

Johnson, Taylor, Keys, and Holt have claimed that animal findings fail to hold for man and that human tropical needs are not different from those of temperate climates. However, all their published data were based upon only a few hours to a few days of heat exposure and provide no just basis for any deductions whatever regarding nutritional needs in the continuous moist heat of tropical lowlands. Human studies along this line must take into account the two- to three-week lag in metabolic acclimatization.

THE NEUROVASCULAR SYNDROME PRODUCED BY HYPERABDUCTION OF THE ARMS

The Immediate Changes Produced in 150 Normal Controls and the Effects in Certain Individuals of Prolonged Hyperabduction as in Sleeping

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(Introduced by Theodore R. Van Dellen, M.D.)

Patients have been observed who have developed numbness, parethesias, trophic changes, and even gangrene of the tips of the fingers as a result of prolonged hyperabduction of the arms. In these patients, the hyperabduction was produced by sleeping or working with the arms in that position. Studies ruled out Raynaud's syndrome, thromboangiitis obliterans, intrinsic and extrinsic tumor of the cervical cord, ruptured nucleus pulposus in the cervical area, infectious polyneuritis, ulnar and median nerve injury, cervical rib and scalenus anticus syndrome.

It was demonstrated that hyperabduction of the arms in the position described resulted in obliteration of the arterial pulse. The question was raised as to whether this constituted a normal or an abnormal finding. Investigation in 150 young adults revealed that obliteration of the pulse could be produced in each arm in approximately 83 per cent of individuals. This could be easily demonstrated in 63 per cent.

The mechanisms playing the most important part are believed to be the stretching of the brachial plexus and the subclavian axillary vessels under the coracoid process with some degree of pinching produced by tightening of the pectoralis minor muscle, and pinching of the vessels and nerves between the

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clavicle and the first rib. Either or a combination of both mechanisms may produce this syndrome. These are not abnormal but normal anatomic arrangements occurring in the majority of individuals but capable of producing a pathologic syndrome after prolonged hyperabduction of the arms. It is to be clearly differentiated from the cervical rib and scalenus anticus syndrome.

DISCUSSION

LIEUTENANT COLONEL FORD HICK, Galesburg, Ill.—I would like to ask whether there was any venous compression.

DR. M. A. BLANKENHORN, Cincinnati, Ohio.—T. Wingate Todd, late Professor of Anatomy at Western Reserve University, produced gangrene of the fingers by tying his arm to the bedpost. Since then none of his colleagues and students has reported that this condition occurs spontaneously. I have not seen it. I wonder whether there was something in the matter of soldiers' training or sleeping that predisposed to this.

COLONEL WRIGHT (closing).—In answer to the first question, the venous compression in a few individuals is quite pronounced; in most it is not an important feature. We find certain individuals in whom the pulse cannot be occluded by this position but who develop paresthesias as a result of pinching of the nerves. In other individuals the obliteration of the pulse seems to be the predominant factor. The veins seem to be able to compensate and to maintain the ability to drain the extremity of blood. There are adequate collateral veins for that purpose, especially with the arterial flow so markedly reduced.

I am very much interested in the observations of Todd. We have been searching the literature for further information in reference to this syndrome and have found very little. The first person to develop gangrene in our series was a civilian who started to sleep with the hands in an hyperabducted position two months before the onset of symptoms. He slept very heavily and never associated his symptoms with sleeping. He went on to the point where he developed gangrene of the tips of the fingers bilaterally. He failed to respond to the usual forms of therapy until the relation to posture was called to his attention. By tying the arms loosely to the bedpost at the foot of the bed he changed his sleeping position and made a rather prompt recovery.

The answer relative to soldiers is very interesting. There are many soldiers who reach a point of exhaustion the like of which they have never encountered in civil life. I have been observing these boys for two years. They come in, throw themselves down in an exhausted state, some with their arms hyperabducted, in which position there is enough pinching to produce paresthesias. If they prolong the pinching the paresthesias may persist the next day. Over a period of time this produces an accumulative effect. Teaching them to sleep with their arms down has freed them of symptomatology but in some instances it has taken as long as three or four weeks.

CARCINOMA OF THE LUNG WITH NONMALIGNANT PLEURAL EFFUSION: RECOVERY BY PNEUMONECTOMY

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The presence of a pleural effusion in association with a bronchiogenic carcinoma is usually considered as a contraindication to successful operation. Tumor cells can be demonstrated in malignant fluids in approximately 50 to 60 per cent of the cases by the section sediment method of Mandelbaum. It is usually assumed, therefore, that even though these cells cannot be demonstrated, such pleural effusions are cancerous and the patient is therefore doomed. That large nonmalignant pleural effusions may occur with carcinoma of the lung is exemplified by the following case.

The patient, a man 50 years of age, had a bronchiogenic carcinoma of the left lung associated with a large left serosanguinous pleural effusion. Study of the fluid showed no tumor cells, but the presence of a moderate number of polymorphonuclears suggested that a secondary inflammatory lesion was probably present. Pneumonectomy was successfully performed by Doctor J. L. Mudd. The lung showed no sign of pleural involvement. The patient shows no evidence of recurrence of the tumor after eighteen months.

THE ASSOCIATION OF PERIARTERITIS NODOSA, BRONCHIAL ASTHMA, AND HYPEREOSINOPHILIA

KEITH S. WILSON, M.D. (By Invitation), AND H. L. ALEXANDER, M.D.
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The triad of periarteritis nodosa, bronchial asthma, and hypereosinophilia was encountered sufficiently often to invite inquiry. Rackemann and Greene in 1939 assembled 237 references to periarteritis nodosa and found twenty-seven cases of bronchial asthma—most of which were accompanied by a hypereosinophilia. We reviewed 300 consecutive cases of periarteritis nodosa proved by tissue section, beginning with the first reported instance of associated asthma in 1914. It is of interest that during the four years of 1940 through 1943, over 150 references to periarteritis nodosa were found with twenty-seven cases of asthma—exactly the same number as in Rackemann and Greene's report that embraced the previous seventy-three years. Thus, there were fifty-four instances of bronchial asthma and periarteritis nodosa in 300 cases, an incidence of 18 per cent. This is doubtless an underestimate since several typical cases were encountered but not proved by tissue section.

Of the fifty-four patients with asthma, the onset of paroxysms antedated the symptoms of periarteritis in all but three. However, the onset of asthma was much later than is usual in that less than 15 per cent began their attacks during the first two decades, whereas some 70 per cent of patients with asthma have their first symptoms before the age of 21 years. In but few cases could extrinsic allergens as foods, pollens, etc., be identified as etiologic factors. As a rule, paroxysms were severe. These facts identify most cases as nonatopic or "intrinsic asthma" as distinguished from the atopic type in which an immunologic mechanism can be identified.

Blood counts were available in forty-seven patients with asthma. Of these, forty-four had a hypereosinophilia ranging from 11 to 84 per cent with an average of 53.5 per cent. This value is far higher than is observed in uncomplicated bronchial asthma. As controls of 151 cases of periarteritis nodosa without asthma in which counts were available, there were nine instances of hypereosinophilia. The average eosinophile count for the group of 151 cases was 2.5 per cent.

It is believed that the association of severe bronchial asthma and marked eosinophilia in an adult should lead to the strong suspicion of underlying periarteritis nodosa.

Present knowledge of the association of periarteritis nodosa with various forms of allergy are commented upon briefly.

DISCUSSION

Lieutenant Colonel Johnson McGuire, Cincinnati, Ohio.—At the Cushing General Hospital near Boston in the past year we saw two extremely interesting cases that in many ways simulate these cases and present a relatively new syndrome. These were soldiers that had come from the Southwest Pacific. They had white counts of 65,000 and 85,000, respectively, with 80 per cent eosinophiles in both instances, and severe asthma. At this time, fortunately for the patients, we had encountered a paper by Kenneth Emerson of the Navy in which this syndrome was described as a tropical eosinophilia and the remedy proposed the administration of arsenic. Muscle biopsies eliminated periarteritis nodosa. There were no other signs of this disorder. There were no parasites present in the stools on repeated examination. Both cases lasted six months and both originated in the same island. In both cases carbarsone was given in small doses for a period of ten days, discontinued for a week and a second course administered. Both cases became free of asthma. The blood count became normal, and they were returned to duty. Twenty similar cases were reported prior to Emerson's publication (Lancet, January, 1943).

Colonel E. V. Allen, Rochester, Minn.—Will Doctor Wilson discuss the method of making a diagnosis in these cases?

Lieutenant Colonel Harold C. Lueth, Evanston, Ill.—At the Research and Educational Hospital we had a patient with intractable asthma in whom a diagnosis of periarteritis nodosa was made. This patient had a marked allergy and was extremely sensitive to many substances.

Dr. C. J. Watson, Minneapolis, Minn.—Hypereosinophilia may disappear suddenly in these patients. We have just recently seen a case exactly of this type, in which the leucocyte count was 65,000 with 60 to 80 per cent eosinophiles. There was epigastric pain such as we see in carcinoma of the pancreas, which was relieved by leaning forward. The diagnosis rested between Hodgkin's disease and periarteritis nodosa. The first biopsy was negative. The second one clearly revealed periarteritis nodosa. After two months this patient suffered what we thought was a thrombosis with perforation, and free gas under the diaphragm, which he survived temporarily. Shortly after the onset of this acute attack his eosinophilia completely disappeared and never recurred. His leucocyte count dropped to 12,000 with 1 per cent eosinophiles. If we had seen the patient during this latter period I am sure we would never have thought of periarteritis nodosa.

Question.—I would like to ask what the incidence of hypertension was in this series of cases.

DR. WILSON (closing).—Lt. Col. Johnson McGuire's remarks on hypereosinophilia and asthma are very interesting. The nature and significance of the eosinophilia is unknown.

The cases of periarteritis nodosa that we studied in the literature were selected because they were proved in practically all instances by biopsy or postmortem findings. Usually biopsy is necessary to clinch the diagnosis of periarteritis nodosa in the living patient.

The patients, needless to say, were critically examined to be sure the asthma was not due to cardiac conditions such as hypertension. We did not analyze the cases for the incidence of hypertension. In a disease that affects the arterial system so extensively, high incidence of hypertension would not be surprising. Elevated blood pressure is found in the patients in whom the renal vessels are extensively damaged. Rackemann and Greene* make no mention of the blood pressure in their eight patients. Harkavy†, who is interested in cardiac manifestations as well as allergy, gives the blood pressure as normal or does not mention it.

It is to be emphasized that the true cause of periarteritis nodosa is not known. Hypersensitivity is offered as one of the mechanisms of its production because of the rather high incidence of allergic phenomena in the cases found in the literature. The triad described is only one syndrome of a disease that produces widespread tissue reaction and vascular damage. The diagnosis in many cases is still dependent on evidences of vascular damage and typical biopsy findings. Nevertheless, the presence of allergic phenomena may be a further clue.

We wish to emphasize the fact, as stated by Dr. Watson, that Henning and Kimball quite a while ago emphasized that repeated differential counts must be made lest the eosinophiles be missed. Lebowitz and Hunt said that an abnormal count of eosinophiles may not come until late in the course of the disease. In other words, the eosinophiles are released in the blood stream and then go to the areas affected. This release may be late. In three of the forty-seven patients with asthma and periarteritis nodosa only one count was recorded. One count had 6 per cent eosinophilia and the other two counts had 4 per cent eosinophilia. But, as stated before, these counts could have been higher during some time in the course of the disease.

DR. M. A. BLANKENHORN, Cincinnati, O.—I was much interested in the last question which was asked and not answered; namely, the incidence of hypertension.

DR. WILSON.—Our paper did not take up the incidence of hypertension in the analysis of these cases. I thought I made that point clear.

METABOLIC EFFECTS OF PARATHYROID HORMONE, DIHYDROTACHYSTEROL, AND CALCIFEROL IN A CASE OF PSEUDOHYPOPARATHYROIDISM

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ROCHESTER, MINN.

A prolonged study of the effects of parathyroid hormone, dihydrotachysterol, and calciferol on calcium, phosphorus, and nitrogen balance was carried out on a patient who presented the clinical features of a condition which Albright has designated as pseudohypoparathyroidism. The metabolic disturbance

*Trans. A. Am. Physicians **54**: 112, 1939.
†J. Allergy **14**: 507, 1943.

in pseudohypoparathyroidism is like that of idiopathic or postoperative hypoparathyroidism but, according to Albright's hypothesis, is due to failure of the organism to respond to parathyroid hormone rather than to lack of the hormone.

The patient, a woman aged 33 years, had experienced convulsive seizures since the age of 14 years. She presented some unusual physical features which were reminiscent of those in the three cases included in Albright's original description of pseudohypoparathyroidism: her habitus was short and thickset; she had a round face and a peculiar deformity of both hands because of partial agenesis of the first, fourth, and fifth metacarpal bones. In addition, there were superficial calcified placques in the subcutaneous tissue of the abdominal wall and legs, and roentgenograms of the head revealed small symmetrical areas of calcification in the region of the basal ganglia and cerebellum. Prior to treatment the value of the serum calcium varied between 4.6 and 5.0 mg. and that of the serum phosphorus between 5.3 and 5.9 mg. per 100 c.c.

Parathyroid hormone in large doses was virtually without effect on urinary phosphorus and on the levels of phosphorus and calcium in the blood serum. Dihydrotachysterol produced the following effects: a rise in serum calcium and a fall in serum phosphorus; a net decrease in fecal calcium and an increase in urinary calcium; a slight net decrease in fecal phosphorus but no change in urinary phosphorus and a more positive balance of both phosphorus and calcium. Calciferol, in the dose employed, had effects which were qualitatively similar to those of dihydrotachysterol but were more marked. None of the therapeutic agents studied produced significant alteration in nitrogen balance. The ratio of calcium to phosphorus in two calcareous placques which were excised from the abdominal wall was 2.07:1, while that in the phalanges of a finger which was amputated because of a longstanding Dupuytren's contracture was 2.12:1.

The data indicate that the patient was resistant to the effects of parathyroid hormone, but that both dihydrotachysterol and calciferol were capable of correcting her metabolic anomaly, mainly by reducing the net excretion of calcium in the feces. The study demonstrates no basic difference in the action of dihydrotachysterol and that of calciferol in this case.

RETINAL HEMORRHAGE AND LENS CHANGES IN ALLOXAN DIABETES IN RABBITS

R. W. Schneider, M.D. (By Invitation), Lena Lewis, Ph.D. (By Invitation), Jacob Moses, M.D. (By Invitation), and E. Perry McCullagh, M.D.
Cleveland, Ohio

We previously reported total and fractional plasma proteins in clinical diabetes mellitus, diabetic acidosis, and diabetic retinitis. Total plasma proteins were determined by the micro-Kjeldahl method and fractional plasma proteins by the Tiselius electrophoretic method. The purpose of this paper is to correlate these findings with those in alloxan diabetes in rabbits.

In uncontrolled clinical diabetes total plasma proteins as well as plasma albumin are usually lowered. After treatment the protein picture reverts to normal as a rule. In diabetes complicated by retinitis, the albumin fraction is

often much lower and is not so easily restored to normal. If control is not sacrificed, higher protein intake improves the protein pattern and in some cases coincidentally tends to prevent hemorrhages.

Alterations in plasma proteins were studied in fifteen rabbits with alloxan diabetes. Eight animals made diabetic with one or two large injections of alloxan maintained normal plasma proteins, and none developed retinal hemorrhage. Five similarly treated animals developed hemorrhages. Two animals that received repeated small injections of alloxan also developed hemorrhages. The animals showing a reduction in total protein and plasma albumin were more apt to develop retinal hemorrhages. The hemorrhages usually appeared within one to three months. The animal observed for the longest time had hemorrhages with associated reduction in plasma albumin on two occasions; a partial recovery in the percentage of albumin was observed after each hemorrhage. After experimental reduction in plasma albumin and subsequent administration of alloxan retinal hemorrhages appeared in from five to seven days. Retinal hemorrhages occurred in one animal in spite of insulin and polyvitamin therapy.

Lens changes were observed in all animals. The most common type of lens change noted was peripheral lens vacuoles. In general, these became more numerous and encroached upon the central area of the lens. The lens changes progressed in direct proportion to the duration of the diabetes. The progression occurred in spite of insulin and vitamin therapy, but the rate of change was retarded.

DISCUSSION

Dr. H. T. Ricketts, Chicago, Ill.—I would like to ask whether retinal hemorrhages were found in the animals deprived of albumin without the production of diabetes. Is it necessary to have an insulin deficiency for these lesions to develop? Second (you may have dwelt on this point but I did not get it if you did), are you able to prevent the development of hemorrhages by feeding the animals a high protein diet before alloxan is administered?

Dr. C. J. Watson, Minneapolis, Minn.—If I understand it correctly, it is your belief that the changes are related principally, but not solely, to low serum albumin. Would you care to comment on the relative infrequence of diabetic retinitis-like changes in conditions such as chronic lipoid nephrosis? I do not believe I have seen changes of the type associated with diabetic retinitis in these cases, even though the serum albumin was at very low levels for long periods.

Dr. Cecil Striker, Cincinnati, Ohio.—I would like to ask if you can correlate the clinical observations of a very severe diabetes in a juvenile who is notoriously devoid of retinal changes and a relatively mild diabetes in a subject past 50 who is notoriously the victim of retinal changes. One is a mild, very easily controlled diabetic with very severe retinal and lens changes, and the other is a very severe diabetic difficult to control with very little retinal or lens changes.

Dr. Schneider (closing).—In answer to Doctor Ricketts, we believe that diabetes must be present first, at least it must be present in addition to a low albumin. We are wondering ourselves whether a high protein diet will prevent these changes. That sort of investigation is going on at the present time. It is interesting in that same connection that two dogs that had been on a high protein diet had no treatment whatsoever and never developed hemorrhage. The rabbits had been on a much lower protein diet than commonly employed in investigation, which may have made them more susceptible to retinal hemorrhage.

Concerning Doctor Watson's comment, I think the answer to that question is that diabetes must be present. We have gone over cases of liver disease, but such individuals do not have retinal hemorrhage.

Doctor Striker, I do not think I can answer your question. I had hoped someone here might have been able to supply that answer.

A TENTATIVE TEST FOR THE DIAGNOSIS OF PHEOCHROMOCYTOMA

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Although paroxysmal hypertension attributable to pheochromocytoma has become a well-recognized clinical entity, its differentiation from coronary occlusion, hyperthyroidism, histaminic cephalgia, migraine, anxiety states, and persistent hypertension may be difficult. Since none of the known methods of inducing attacks is dependable and since opportunity to observe the patient in a spontaneous attack may not present itself, a simple procedure that would induce attacks at will in these cases would be of great help in diagnosis. The response of fifty-one persons to intravenous injection of 0.05 mg. of histamine base suggested that this method might be used as a clinical test.

Four groups of persons were studied: in Group 1 were nine normal subjects; in Group 2, twenty-two hyperreactors to the cold pressor test; in Group 3, sixteen patients with well-established hypertension; in Group 4, four patients who were suspected of having pheochromocytomas.

In Groups 1 and 3 flushing of face and subsequent headache followed intravenous injection of histamine. These symptoms were mild and disappeared in from five to ten minutes except in one instance in which typical histaminic cephalgia developed. The average elevation of blood pressure was identical with that during the cold pressor test in Group 1 and was 12 mm. of mercury systolic and 15 mm. diastolic less than that during the cold pressor test in Group 2. In Group 3 circumoral pallor appeared often and headaches frequently were severer and lasted longer. The average rise of blood pressure was 6 mm. systolic and 9 mm. diastolic less than that during the cold pressor test.

Three of the four patients in Group 4 had attacks within two minutes after injection. These were characterized by severe headache, distress throughout the abdomen, and later in the chest, groaning, refusal to stay on the bed, and circumoral pallor with subsequent excessive sweating. The systolic blood pressure rose from levels of 110 to 132 mm. to levels of 240 to 260+ mm. while the diastolic pressure rose from levels of 68 to 85 mm. to levels of 142 to 146 mm. In one case these heights were attained after injection of only 0.025 mg. of histamine base. The cold pressor, insulin, and epinephrine tests did not induce attacks in these three patients. At operation, pheochromocytoma was found in the adrenal medulla of each of the three patients. After operation, when the histamine test was repeated, response was normal. The fourth patient in Group 4 responded normally to histamine and operation did not disclose a tumor.

Although the number of cases studied is small, the intravenous injection of small quantities of histamine base may be considered tentatively as a clinical

test in distinguishing the syndrome of pheochromocytoma from other clinical conditions.

DISCUSSION

WALTER F. KVALE, Rochester, Minn.—Up to the present time we have carried out this test in forty-two patients in whom it was considered necessary to differentiate paroxysmal hypertension as the result of a pheochromocytoma. The test was considered negative in all patients and in four of these in whom an opportunity was presented for exploration of the adrenal regions, no tumor was found.

During the past seventeen months we have had the opportunity of observing three patients in whom a correct clinical diagnosis of pheochromocytoma was made. The rapid injection of small amounts of histamine in each of these three patients produced an attack identical with the spontaneous attacks of which the patients complained and afforded us the opportunity of observing them in their attacks at such times as we wished. An attack was induced at will five times in one patient, twice in another, and once in a third. In each attack there was a marked rise of both the systolic and diastolic blood pressure which promptly returned to basal levels within ten minutes when the attack subsided.

DR. B. T. HORTON, Rochester, Minn.—In 1939, at the meeting of this Society, Dr. H. L. Alexander gave me the idea of giving histamine intravenously, as a therapeutic agent. Since that time we have given 25,821 such injections, 13,400 of which have been given since I discussed the subject before this Society a year ago.

It was because of my interest in histamine that Doctor Roth asked me to see Patient 1, with paroxysmal hypertension, after she had failed to precipitate attacks by means of the cold pressor test of Hines as well as by the use of insulin and adrenalin.

I attempted to give the patient histamine intravenously by the drip method, using a 1:250,000 dilution according to our standard method. Histamine promptly precipitated an attack. The blood pressure, which Doctor Roth recorded, rose to a high level, as she has indicated. After a lapse of time I gave the same patient 0.05 mg. of histamine intravenously, and an attack developed within forty seconds, with a resulting rise in blood pressure from 130/110 to 280/295. I made a technicolored movie of the attack, which a number of the men in this audience have seen. Following surgical removal of the pheochromocytoma, histamine no longer precipitated attacks.

Why does a paradoxical rise in blood pressure occur following the injection of histamine intravenously? Histamine and adrenalin are physiologic antagonists. Schenk, in 1921, was apparently the first to call attention to this fact. A minute amount of histamine when given intravenously in such a case causes a sudden outpouring of adrenalin into the blood stream. A pulsating type of headache develops immediately due to the sudden rise in blood pressure. This is the basis of the syndrome of paroxysmal hypertension. This headache need not be confused with histaminic cephalgia, because it is generalized, whereas histaminic cephalgia is always unilateral.

Histamine can be used intravenously to bring out localizing signs and symptoms of other obscure tumors. I would like to refer briefly to the case of a woman aged 31 years with symptoms of two and a half years' duration, suggesting multiple sclerosis. During the course of twenty-two intravenous injections of histamine her symptoms became markedly worse. Signs of a cord lesion at the level of the tenth dorsal vertebra developed, and surgical exploration Oct. 17, 1944, revealed an intramedullary tumor 2 by 1½ cm. which was removed. She is now convalescing.

How did histamine act in this case? It caused a rise in intraspinal pressure, thus exaggerating the pressure symptoms of the tumor to the extent that the obscure lesion could be diagnosed clinically.

DR. H. NECHELES, Chicago, Ill.—We should be interested in the mechanism through which this effect is produced in cases of pheochromocytoma, and I wonder whether Doctor Roth has any suggestions. It may be a carotid sinus effect, and the same reaction may be obtained with other procedures affecting the carotid sinus.

Doctor Roth mentioned an amount of 50 to 1,000 mg. of adrenalin in the tumor. Is that in actual milligrams?

DR. ROTH (closing).—There has been some experimental work done by Kellaway and Cowell who injected histamine intravenously into a nonetherized cat and observed transitory slowing of the heart, dilatation of the pupils, and sweating. In an animal with intact adrenals, an injection of histamine produced dilatation of the denervated pupil. In an animal from which the entire adrenals or the medulla alone had been removed, the histamine caused no such dilatation. This was considered proof that histamine accelerated a secretion from the medulla of the adrenal glands.

In regard to the carotid sinus, Hyman and Mencher found that massage of the carotid sinus during the maximum rise in blood pressure caused a prompt decrease in the blood pressure which immediately rose when massage was stopped.

It was in actual milligrams.

ISOIMMUNITY TO THE RH FACTOR AS A CAUSE OF BLOOD TRANSFUSION REACTIONS

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IOWA CITY, IOWA

Ever since the demonstration of isoimmunity to the Rh factor, incurred during pregnancy or after multiple blood transfusions, it has been difficult to obtain a proper perspective on the role of this phenomenon in the production of transfusion reactions in a general hospital service. During a period of eighteen months the blood transfusions in the State University of Iowa Hospitals were studied with respect to this problem. Suitable tests for anti-Rh agglutinins were made on the blood of all recipients exhibiting chills, fever, hemoglobinuria, or jaundice after transfusion. In 5,386 consecutive blood transfusions there were 186 reactions of all types, an incidence of 3.4 per cent. Of these, six were due to isoimmunity to the Rh factor, an incidence of 0.1 per cent in all transfusions, many of which were given to the same recipient or to pregnant women. In four cases isoimmunity was attributable to multiple transfusions and in two, pregnancies were responsible. One fatality occurred.

CASE 1.—A male physician, 37 years of age, after a gastric resection had three transfusions in one month without reaction. Two months later another transfusion produced chills, fever, and bilirubinemia. His blood proved to be Rh negative but no anti-Rh agglutinin could be demonstrated.

CASE 2.—A man 21 years of age had tuberculosis of the hip. He received ten blood transfusions in a month, the last few of which were accompanied by chills and fever. His blood was found to be Rh negative and contained an anti-Rh agglutinin. Two subsequent transfusions with Rh-negative blood were given without reaction.

CASE 3.—A woman aged 37 years received three blood transfusions without reaction in one week for an incomplete abortion. A fourth transfusion

after an interval of twenty-one days produced intravascular hemolysis. Her blood was found to be Rh negative and contained an anti-Rh agglutinin. Subsequent transfusion with Rh-negative blood produced no reaction. She had eight living children whose bloods were typed for the Rh factor. There had been no manifestations of erythroblastosis in any of her pregnancies.

CASE 4.—A woman aged 49 years was treated for metrorrhagia with a blood transfusion which was followed by chills and fever. Another transfusion was given the next day which resulted in hemoglobinuria and jaundice. Her blood was found to be Rh negative and contained an anti-Rh agglutinin of extremely high titer. Three subsequent transfusions with Rh-negative blood gave no reactions. Seven pregnancies had occurred more than ten years before, resulting in three living children, three abortions, one stillbirth, and one infant dying from umbilical hemorrhage.

CASE 5.—A woman aged 25 years was given a blood transfusion for toxemia of pregnancy. Chills, fever, dyspnea, bilirubinemia, and bilirubinuria followed, but at no time was there free hemoglobin in the blood stream. A dead fetus was promptly delivered which had the typical stigmas of erythroblastosis. The patient's blood was Rh negative and that of the baby was Rh positive, but no anti-Rh agglutinin could be demonstrated in the mother's blood. Two weeks later such an agglutinin had appeared.

CASE 6.—A woman aged 30 years had never been pregnant. Eleven years previously she had received three blood transfusions and a year later, two more, in the course of a lobectomy and the drainage of a subphrenic abscess. There had been no reactions. In July, 1944, another transfusion was given without reaction. Twelve days later another blood transfusion was followed by chills, fever, and hemoglobinuria. Oliguria ensued and, although decapsulation of the kidneys was performed on the third day, she died on the twelfth day in uremia. Her blood was found to be Rh negative and contained an anti-Rh agglutinin which reacted with the Rh-positive blood which she had received.

Conclusions.—1. Isoimmunity to the Rh factor was detected in 0.1 per cent of 5,375 consecutive blood transfusions and accounted for only 2.4 per cent of the reactions of all types which were noted in the series.

2. Routine compatibility tests for the detection of an anti-Rh agglutinin would have prevented four out of the six reactions reported in this series. They would have prevented one fatality in over 5,000 blood transfusions. These tests will not prevent all reactions due to isoimmunity to the Rh factor.

3. It is recommended that suitable compatibility tests for the detection of the Rh factor be performed before transfusion of any recipient who has ever been pregnant or has received transfusions more than one week previously.

DISCUSSION

DR. C. C. STURGIS, Ann Arbor, Mich.—I should like to ask Doctor DeGowin what he means by "routine compatibility tests" and also have him amplify his statement that tests for Rh agglutination would have prevented four out of the six reactions. I assume he wished to indicate that the blood of both the recipient and the donor should have been typed for the Rh factor and Rh-negative blood should then have been given to the Rh-negative recipient. May I also

ask him to express his opinion about the effectiveness of the compatibility tests with respect to the Rh factor in which the red blood cells of the donor are brought in contact with the recipient's serum at room temperature and when they are incubated at 37° C. for one hour.

DR. C. J. WATSON, Minneapolis, Minn.—If one carries out the hemolysis test for an hour in the incubator, would that be sufficient to exclude a reaction of this type?

DR. W. J. DIECKMANN, Chicago, Ill.—I wish, as an obstetrician, to endorse Doctor DeGowin's comments about the importance of the Rh blood group, especially, as I am a convert.

We recently had two Rh-negative patients, but without any demonstrable anti-Rh agglutinins, who had given birth to babies with erythroblastosis, and gave 100 c.c. of Rh-positive blood to one and 50 to another. Each patient had a severe reaction and increased bile pigments in the serum.

Within the past month an Rh-negative patient was given two Rh-positive blood transfusions because no Rh-negative blood was available. In such cases sodium bicarbonate should be given in 4 Gm. doses every two hours for twelve hours, or if the patient is in shock, 300 c.c. molar sodium lactate dissolved in 2,700 c.c. 5 per cent glucose should be given half intravenously and the remainder by clysis.

DR. DEGOWIN (closing).—With regard to Doctor Sturgis' question about compatibility tests, there is a distinct difference in the way antiserum containing Rh agglutinins reacts in the ordinary laboratory tests and the reactions of naturally occurring isoagglutinins. In performing cross-matching of blood with regard to the ABO system, no incubation is required. Any procedure which brings the agglutinins and agglutinogens in contact and then produces sedimentation is perfectly satisfactory. In looking for the anti-Rh agglutinins, it is apparently necessary to incubate the antiserum with the cells at 37° C., usually for a period varying from twenty to sixty minutes. The procedure also differs in the care which must be taken in manipulating cell suspensions to prevent excessive shaking. Ordinarily, with anti-A and anti-B sera, the resulting cell clumps cannot be shaken apart. On the other hand, clumps formed by anti-Rh sera are frequently small and are readily broken up by agitation so that the reaction may be entirely missed. The performance of tests involving anti-Rh agglutinins is difficult and requires much more experience than when natural agglutinins are concerned. Even as at present performed, agglutination may be easily overlooked.

With regard to Doctor Watson's question, searching for hemolysis in the test tube is inadequate to demonstrate incompatibility to the Rh factor. Although hemolysis occurs when incompatible blood is introduced into the body, no one has succeeded in demonstrating hemolysis in the test tube in this type of incompatibility.

Another clinical fact which has not been dealt with in this paper is that the tissues of the baby with erythroblastosis fetalis contain the anti-Rh antibodies of the mother. If the baby, whose blood is Rh positive, is transfused with more Rh-positive blood, the transfused cells are quickly hemolyzed. The child should receive Rh-negative blood.

EXPERIMENTAL STUDY OF ANURIA IN BURNS

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We have reported previously that the anuria occurring in shock can be classified into three different types. The anuria following thermal trauma begins immediately after the burn. The present report deals with a comparison of various intravenous fluids used in order to overcome this acute anuria.

Animals were used under permanent anesthesia; all experiments were acute. The degree of hemoglobinemia was the most reliable index of the severity of the anuria.

Plasma and gelatin had very little therapeutic value in restoring urine secretion. The following solutions have some value: lactate plus 5 per cent glucose and 2 per cent urea; saline plus 2 per cent urea; Tyrode's solution; and saline with .87 per cent sodium bicarbonate. Isotonic sodium sulfate has proved to be superior to most fluids in overcoming the anuria due to burns.

DISCUSSION

DR. NECHELES.—We believe that the free plasma hemoglobin following burns is an indication of the severity of the burn. In the experiments reported by Mr. Olson, we can be sure that the dogs with very high values for free plasma hemoglobin were the more severely burned, and we have grouped our experiments accordingly. It is interesting that in the reports on burns in human beings most authors do not report on free plasma hemoglobin. We know that hemoglobin is quickly taken care of by the reticulo-endothelial cells and by the kidneys. We feel that the concentration of free plasma hemoglobin is related to the degree of kidney damage. Experiments to determine this are under way. So far, sodium sulfate seems to be the best solution to overcome the anuria of burns in acute experiments.

THE DISTINCTION OF HEMOLYTIC AND NONHEMOLYTIC TRANSFUSION REACTIONS

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MINNEAPOLIS, MINN.
(INTRODUCED BY W. W. SPINK, M.D., MINNEAPOLIS, MINN.)

Transfusions of whole blood or red blood cells may be attended by reactions. The majority of these are simple febrile or urticarial types, but some are hemolytic. The recognition of hemolytic transfusion reactions and their distinction from those of simple febrile type are often matters of considerable practical importance.

The present study of transfusion reactions has depended upon the quantitative determination of hemoglobin in blood plasma and urine. Artificial hemolysis was prevented by drawing the blood carefully into isotonic sodium citrate solution. The hemoglobin concentration of the plasma was determined by the method of Flink and Watson, in which hemoglobin is converted to pyridine hemochromogen and measured by means of an Evelyn photoelectric colorimeter. Normal values with this method range from a trace to 5 mg. per 100 c.c. of plasma. With hemoglobin concentrations of from 20 to 25 mg. per 100 c.c. the presence of hemolysis is evident to the eye.

Nineteen nonhemolytic febrile reactions were studied. Seventeen of these followed whole blood transfusions and two followed plasma infusions. Some of these reactions were very severe, with high fever and chills lasting up to thirty minutes. Blood specimens were obtained within the hour after the reaction. The highest plasma hemoglobin concentration was 12 mg. per 100 c.c. and the remainder was less than 9.0 mg. per 100 c.c. Since some of the patients were critically ill, the absence of significant hemolysis was a finding of considerable prognostic and therapeutic importance.

Five hemolytic transfusion reactions in as many patients were studied during a two-year period. One patient who received multiple transfusions during a craniotomy developed anuria and died seventy-six hours later. Multiple transfusions of Group 0 uncross-matched blood had been given during the operation. Twenty-four hours after the first transfusion only oxyhemoglobin was present on spectroscopic examination of the plasma, but at forty-eight hours and sixty-eight hours methemalbumin (Fairley) as well as oxyhemoglobin were found. Two patients had hemoglobinuria and hemoglobinemia after receiving blood during operations but recovered uneventfully. Plasma specimens were obtained at the time of subsidence of the hemoglobinuria in each instance so the concentration of hemoglobin was relatively small (54 mg. per 100 c.c. plasma in one and 38 mg. per 100 c.c. in the other patient). In two patients the hemolysis was recognized by finding 38 mg. hemoglobin per 100 c.c. plasma in one and 125 mg. hemoglobin per 100 c.c. plasma in the other; hemoglobinuria did not occur in either patient. All five patients became jaundiced on the day following the reaction, thus substantiating the hemolytic nature of the reactions.

There are many reports of serious or fatal transfusion reactions with oliguria or anuria for a time, but very little mention is made of the degree of hemoglobinemia or of the degree of hemoglobinuria. It has been suggested that the presence or absence of renal damage is related directly to the degree of hemoglobinemia. The absence of renal damage in most patients with paroxysmal nocturnal hemoglobinuria is probably related to the relatively small concentration of hemoglobin in the plasma at any one time. Evidence for this will be presented.

The importance of immediate determination of the presence or absence of abnormal amounts of hemoglobin in the plasma following febrile transfusion reactions is stressed. Unless hemoglobinemia and hemoglobinuria are looked for, it is impossible to distinguish hemolytic from simple febrile reactions on clinical grounds alone. If alkalinization of the urine is to be considered in patients with transfusion reactions, it is reasonable to determine whether or not hemolysis has occurred before pursuing such therapy.

DISCUSSION

Dr. Elmer L. DeGowin, Iowa City, Iowa.—I should like to endorse everything that Doctor Flink has said and also ask him a question. In the case of the patient he reported with anuria, was the method of determining the hemoglobin in the blood serum such as to differentiate it from methemoglobin? I have in mind the work of Bing on animals where he could not produce renal lesions by the intravenous injection of hemoglobin. In the case of anuria which I just reported, a blood sample from the recipient, taken fourteen hours after the transfusion reaction, contained hemoglobin in the serum but no methemoglo-

bin. There is still a good deal of discussion concerning the damage which hemoglobin can inflict on the kidney. I should like to hear Doctor Flink's comment on that.

DR. J. S. LADUE, New Orleans, La.—Were any studies made on urobilinogen excretion to see whether the hemoglobin was secreted through the kidney or through the gastrointestinal tract? Second, did hemoglobinuria produce any other evidence of liver dysfunction?

DR. C. J. WATSON, Minneapolis, Minn.—I would like to comment upon the transition of hemoglobin to bilirubin in the circulating blood and reticulo-endothelial system. It is commonly believed that hemoglobin is converted to bilirubin only in the reticulo-endothelial system. There is, however, a growing body of evidence that the conversion may also take place in the circulating blood under what may be called relatively normal circumstances. If a large amount of hemoglobin is liberated in the circulating blood, there may be resultant liver damage. As evidence of this the van den Bergh reaction that has been indirect becomes promptly direct in part, and a large amount of urobilinogen appears in the urine. With the more severe reactions, bilirubinuria may also be noted. In such instances hematin (methemalbumin) is likely to be observed in the serum. This is quite characteristic of severe hemolysis plus hepatic damage, as, for example, in blackwater fever. It appears that when the liver is damaged, some of the hemoglobin is converted to hematin rather than bilirubin, as contrasted with the sequence of events when one injects small amounts of hemoglobin into the circulation.

DR. FLINK (closing).—In answer to Doctor DeGowin, there was no methemoglobin in the plasma of the last patient. I looked for it each time the plasma was obtained for examination. People are not generally aware of the occurrence of methemalbumin which has an absorption band at 623 to 624 mu. It has been confused in the past with methemoglobin. Fairley has made the statement that during hemoglobinuria in blackwater fever methemoglobin never occurs in the plasma. There is no methemalbumin in the urine either. As to the mechanism of renal damage, I agree that it is not well understood at the present time.

In answer to Doctor LaDue, no urobilinogen tests were carried out either in the hemolytic reactions or in the febrile nonhemolytic reactions. It would have been well to do so. In patients with uncomplicated transfusions there is evidence of increased urobilinogen excretion when blood older than five days is used as compared to instances where fresh blood is used. As to liver damage I have no comment. It is interesting that the patient with paroxysmal hemoglobinuria had methemalbuminemia constantly; that may be an index of liver dysfunction.

DR. C. J. WATSON, Minneapolis, Minn.—I would like to say that if methemoglobin were present it would be included. Doctor Flink's statement about looking for methemoglobin separately is the only way it can be excluded. We have studied the urobilinogen in the feces and marked increases have been observed. A considerable amount of hemoglobin is converted into bile pigment and thence to urobilinogen.

FACTORS CONTROLLING THE CORONARY CIRCULATION

LOUIS N. KATZ, M.D., WILFRED WISE, M.D. (BY INVITATION), AND KENNETH JOCHIM, PH.D. (BY INVITATION)
CHICAGO, ILL.

For the past dozen years we have been investigating the control of the coronary circulation on preparations in which the coronary vessels were perfused from a separate circuit. In the intact animal, however, the coronary vessels are perfused from the same trunk, the aorta, which feeds the other systemic circuits. For this reason, we have recently analyzed seventy-nine experiments on the isolated denervated heart and heart-lung preparations in which this normal parallel circuit arrangement was permitted to exist. In these experiments, the coronary flow was measured by the difference in flow between the main pulmonary artery and the aorta simultaneously determined with modified Ludwig flowmeters.

The coronary flow was found to depend upon the cardiac output per minute, often independently of the aortic pressure level. This correlation between cardiac output and coronary flow of the denervated heart is explained on a purely mechanical basis. When the output of the left ventricle increases, blood is forced at an increased rate through both the coronary and the other systemic circuits. This increased flow tends to distend these circuits, thereby reducing their resistance, since resistance in a system of elastic tubes is an exponential function of vessel caliber. This automatic decrease in resistance may be sufficient in many instances to prevent any significant rise in aortic pressure.

In addition, with the cardiac output constant, changes in the peripheral resistance of the extracoronary systemic circuit caused parallel changes in coronary flow, sometimes without any significant alteration in the aortic pressure. This change in coronary flow is determined by changes in the resistance of the extracoronary paths per se. The total peripheral resistance may not change appreciably if the coronary resistance is, at the same time, sufficiently decreased by the passive distention caused by the diversion of blood to the coronary bed, so that aortic pressure may not change appreciably.

Thus, in the intact circulation coronary flow depends primarily on two passive factors: (1) the cardiac output per minute and (2) the state of constriction of the extracoronary systemic blood vessels. Furthermore, changes in these two factors can modify coronary flow independently of obvious arterial blood pressure changes.

These facts, of course, do not negate the value of powerful coronary vasodilator drugs to supplement such mechanical (and other possible humoral) regulators. However, in employing such drugs care should be taken (1) that they are powerful enough, (2) that they do not at the same time increase the work of the heart proportionately (or out of proportion), and (3) that they do not divert blood from the coronary circuit by an equal or greater dilating action upon the other systemic vascular beds.

DISCUSSION

DR. JOHN R. SMITH, St. Louis, Mo.—I was rather cheered to hear Doctor Katz say that he was shocked at some of his results, because I ran into that in

some of my experiments. I started out to perfuse one of the auricular arteries and to study perfusion into the auricular arteries under ordinary tension. In noting the perfusion of blood into the left anterior auricular artery, I found that by raising the cardiac output without changing the blood pressure, inflow into this little artery increased three or four times. Fearing that something was wrong in my experiments, I repeated them three or four times. Apparently the same thing as Dr. Katz noted with the total coronary artery inflow was what I noticed with only one little twig of the coronary system.

Dr. Emmet B. Bay, Chicago, Ill.—I would like to ask Doctor Katz what he thinks the mechanism is that makes the dilatation in the coronary arteries. Something has to dilate the coronaries and possibly that very slight increase in pressure would serve to dilate them. In the other experiment it is also quite clear, as Doctor Katz pointed out, that if carried to absurdity the vessel would have ruptured; this in Doctor Katz mind must mean quite an increase in pressure. I do not believe it is fair to talk in terms of constriction at the root of the aorta determining coronary flow without a change in pressure since other work has quite clearly demonstrated that by changing the pressure at the root of the aorta it is possible to change the coronary flow.

Dr. Moses Barron, Minneapolis, Minn.—I would like to ask Doctor Katz if the coronary flow is increased in cases of coarctation of the aorta.

Dr. Katz (closing).—I am delighted to hear that Doctor Smith had a similar experience on perfusing a single coronary artery.

Doctor Bay's point is well taken. In my presentation, I was careful to state that there was no appreciable change in aortic pressure. We do not deny that the pressure rose, but the rise was often tiny, less than 1 mm. of mercury, despite the large augmentation of the coronary flow. Such a rise in blood pressure is of no importance clinically and would be considered as no change. Furthermore, the pressure change in the aorta is no criterion of the pressure change in the smaller coronary vessels. Even if the aortic pressure changes little, it is still possible that the pressure in the smaller vessels will rise appreciably. Distention of the coronary vessels will cause the pressure gradient to fall; therefore, if the aortic pressure does not rise significantly the pressure in the small coronary vessels should on this account rise definitely. Our argument is thus directed against the idea that aortic blood pressure change is the sole factor which alters coronary flow passively. We agree, and have ourselves demonstrated, that coronary flow increases when the aortic pressure goes up, but in the intact animal we insist that coronary flow may increase greatly with practically no change in aortic pressure.

As to Doctor Barron's question about coronary flow in coarctation of the aorta, I would be inclined from our experiments to believe that the coronary flow is definitely increased because the coarctation is equivalent to the screwing down of the screw clamp on the aorta of our heart-lung preparation. When the coarctation is small, there may be no rise in aortic pressure proximal to the lesion and yet the coronary flow will be increased. With more severe lesions the pressure proximal to the lesion will also go up.

THE USE OF PAPAVERINE IN CORONARY ARTERY DISEASE

L. W. Swanson, M.D.
Mason City, Iowa

Since the very favorable report by Katz on the efficacy of papaverine in large dosage in the treatment of angina pectoris, twelve patients with varying degrees of coronary insufficiency have been treated with papaverine. Their ages varied from 42 to 74 years. Four of the twelve had angina of effort but no evidence of coronary occlusion either by history or electrocardiographic examination, while the remaining eight all gave a history of healed coronary occlusions which had been confirmed by observation and/or typical changes in the electrocardiographic pattern. The duration of symptoms varied from two months to one year. All of the patients complained that their respective activity levels were much reduced by the anginal pains; three of them had actually been bedridden by the severity and frequency of the attacks. Papaverine hydrochloride was administered orally in doses of 1½ gr. four times daily.

Eleven of the twelve patients appeared to be definitely improved by the drug. One did not, and the administration of the drug had to be stopped because of excessive sleepiness. In none of the other cases was oversedation noted as a disturbing side-effect. No suggestion of addiction was observed upon cessation of the treatment. Two of the bedridden patients were improved enough to tolerate the activity of being up enough to care for themselves at home after approximately three and five weeks of treatment, respectively. Two of the others who had been forced by their attacks to quit all manual labor were able to resume light work after one and three weeks of papaverine administration. The remainder of the eleven patients who improved measured their improvement by stating they had fewer attacks of pain and could walk farther.

These results are thought to be significant even in a small series because of the high proportion who were improved and because all of the patients had previous therapeutic trials on conventional methods.

DISCUSSION

Dr. Moses Barron, Minneapolis, Minn.—I would like to ask the essayist how long he keeps up this treatment of 1½ gr. four times a day.

Dr. Swanson (closing).—One patient has been on this treatment almost continuously for fourteen months. Whenever he gets into trouble he wants to resume it.

STUDIES ON THE CHANGES IN THE CIRCULATION OF NORMAL SUBJECTS WITH SMALL VARIATIONS IN THE FLUID CONTENT OF THE BODY

Richard H. Lyons, M.D., Franklin D. Johnston, M.D., and John Sanders, B.S. (By Invitation)
Ann Arbor, Mich.

The administration of 2 c.c. of mercupurine to ten normal subjects resulted in a mean loss of weight of 1.76 ± .3 kg., a decrease in the plasma volume of 544 ± 88 c.c., and a decrease in venous pressure of 25 ± 5 mm. of water. The

significance of these changes was further studied in seven other normal subjects on whom twelve observations of the cardiac output, stroke output, and heart rate were made with the ballistocardiogram twenty-four hours after a 2 c.c. injection of mercupurine. These observations were compared to thirty-five control studies. There was a mean decrease in the stroke volume of 17 per cent; in the cardiac output per minute, of 12.4 per cent; in the venous pressure, of 15.8 per cent; and in body weight, of 1.9 per cent. The heart rate increased 9.5 per cent and there was an 8.2 per cent increase in the diastolic pressure. These changes were statistically significant when compared to the control observations. Some subjects complained of weakness, tiredness, and faintness on standing.

The oral administration of large amounts of sodium chloride (20 Gm.) or sodium bicarbonate (25 Gm.) is associated with an increase in plasma volume, 400 to 440 c.c., a rise in venous pressure of 17 to 30 mm. of water, and a gain in body weight of 1.6 to 1.9 kg. Eleven observations on seven normal subjects after the ingestion of 25 Gm. of sodium chloride per day for two days showed a mean increase in stroke output of 9.4 per cent; in cardiac output, of 4 per cent; in systolic blood pressure, of 7.2 per cent; in venous pressure, of 28.8 per cent; in body weight, of 2.2 per cent. There was a decrease in the heart rate of 5.3 per cent. Except for the increase in cardiac output and decrease in heart rate, these changes were statistically significant when compared to the control observations.

It is concluded that small changes in the extracellular fluid content of the body of 1.5 to 2 liters is sufficient to produce changes in the plasma volume, venous pressure, stroke output, and pulse pressure.

DISCUSSION

Dr. G. T. Evans, Minneapolis, Minn.—Has there been any observation made on increasing the intake of plain water? I am thinking particularly of the experiment of Margaria in which a larger intake of fluid was not accompanied by a dilution of the blood.

Question.—May I ask if Doctor Lyons has any observations on the hematocrit, hemoglobin, or the plasma-protein of the blood.

Dr. Lyons (closing).—In measuring plasma volume we have also done hematocrits and have found in general that percentage change in plasma volume determined by the dye method may be considerably more than the percentage of change of the hematocrit. The hematocrit has not been a good index of change in the plasma volume. The same is true of the serum protein concentration which does not change as much as the plasma volume determined by the dye method. This may be due to the change in total circulating protein. Roughly, when we have an increased plasma volume, we get an increase in the circulating protein, and with a decrease in plasma volume there is a decrease in total circulating protein; therefore the change in protein concentration will not vary as much as the change in plasma volume.

We have made no experiments on the viscosity of the blood so I cannot comment on that.

Regarding the change in plasma volume with the ingestion of large volumes of water, we have observations on medical students who have ingested 2 liters of water and we have found significant change in the plasma volume.

DEVELOPMENT OF A TEST FOR THE DETECTION OF ANTIBODIES IN SERUM OF TUBERCULOUS HUMAN BEINGS AND GUINEA PIGS

R. O. Muether, M.D., and William C. Macdonald, M.D. (By Invitation)
St. Louis, Mo.

The serologic diagnosis of tuberculosis has always been unsatisfactory, but the desirability of having a satisfactory procedure has never been questioned.

Cannon and Marshall, using collodion pellets coated with old tuberculin, have devised a technique which has been useful in their hands. We have been interested in this problem for a number of years and have attempted to develop a satisfactory technique basing our work on that of Roberts and Jones with virus agglutination tests.

The technique used at present consists of coating a nonpathogenic organism, *Serratia marcesens*, with old tuberculin. To secure a satisfactory antigen, it has been found necessary to have a uniform suspension of organisms of constant density and it is important that the ratio of bacterial N to old tuberculin nitrogen be adjusted carefully.

Once a satisfactory antigen is prepared, the antibodies, if present in serum, will cause precipitation and the titer of the antibodies can be determined by making serial dilution of the serum.

In the present study, infected guinea pigs as well as human subjects were used.

The guinea pigs were infected with suspensions of tubercle bacilli and then bled at four-day intervals for twelve days. Each serum was tested for antibodies. The serum of the infected animals gave positive tests in increasing titer as the infection progressed, while the control animals remained normal.

Studies on human sera have shown negative reactions when applied to "tuberculin-negative" individuals, while "tuberculin-positive" patients without active tuberculosis showed a weak reaction (1:64). Active cases of pulmonary tuberculosis gave high titers up to 1:1024. Arrested cases showed much lower titers ranging from 1:32 to 1:256.

TOXICITY OF SALICYLATES*

Major Frederick S. Coombs, Major Harry A. Warren (By Invitation), and Lieutenant Colonel Charles S. Higley (By Invitation)
Medical Corps, Army of the United States

Recent papers have advocated the establishment of plasma salicylate levels of between 30 to 40 mg. per 100 c.c. without the use of sodium bicarbonate in the treatment of acute rheumatic fever.

We have studied a series of eighty-four patients with acute rheumatic fever in whom the plasma salicylate levels have been followed during intravenous and oral administration of from 10 to 16 Gm. daily. While it is too early to evaluate the efficacy of such treatment in preventing cardiac lesions, we have been impressed with the toxic reactions occurring in such patients when plasma salicylate levels are maintained at over 30 mg. per 100 c.c. Previous investigators

*From Truax Field, Madison, Wis.

have reported partially on this syndrome, but before salicylate levels were available. Re-emphasis now is deemed important in view of current interest in salicylate therapy in acute rheumatic fever.

The toxic reactions, or salicylism, may be divided into three parts:

1. Respiratory alkalosis and its concomitant changes
2. Pustular acne
3. Maniacal delerium

The respiratory alkalosis is characterized clinically by an increase in the rate and depth of respiration, at times severe enough to cause numbness and tingling of the acral parts and on one occasion to produce carpo-pedal spasm. Chemically the blood shows a rise in pH, a lowering of CO_2 content, rise in serum chlorides, early compensatory loss of sodium, and retention of water. Decreased renal function measured by urea clearance and intravenous phenolsulfonephthalein tests results. Charts demonstrate these changes.

When sodium bicarbonate is administered along with sodium salicylate, lower blood levels are obtained, and the respiratory alkalosis does not develop principally because sodium bicarbonate increases the excretion of salicylates.

Colored slides illustrate the pustular acne which simulates bromidism. The effect on the central nervous system is outlined.

The theory that accumulation of salicylates in the blood leads to direct stimulation of the respiratory center and thus to hyperventilation alkalosis is propounded. Continued alkalosis of the degree observed in some of our patients is severe enough to interfere with kidney function and may lead to permanent renal damage.

DISCUSSION

Dr. Walter L. Palmer, Chicago, Ill.—The renal changes are not proof of renal injury. Doctor Kirsner, in his study of alkalosis, did not find definite evidence of renal damage. The changes in function return to normal when the electrolyte balance becomes normal. In other words, the alteration in renal excretion may be purely a reflection of the disturbed electrolyte balance.

Dr. A. J. Quick, Milwaukee, Wis.—I think that these new clinical studies employing large doses of salicylates should stimulate further investigation concerning the metabolism of salicylic acid. It is to be remembered that salicylic acid is really a phenol with the properties of the phenolic group modified by the carboxyl group. Salicylic acid is slowly eliminated from the body—even after taking only 1 Gm. of sodium salicylates, salicylic acid can be detected in the urine for twenty-four hours and often for forty-eight hours. We know that salicylic acid undergoes various metabolic changes in the body. Some of it is combined with glycine; some is oxidized to a dihydroxy salicylic acid. We do not know much about these polyhydroxy compounds, but it is probable that they may exert a toxic action on bone marrow and nerve tissue. Since salicylates are now used in massive doses, it might be well to investigate further the fate of salicylic acid in the organism. Coburn has done some of this work but more study is necessary.

Dr. C. J. Watson, Minneapolis, Minn.—Did you follow the prothrombin or fibrinogen levels in these patients?

Major Coombs (closing).—We, of course, were interested in knowing whether the prothrombin time was changed. We had some patients with a blood salicylate level of approximately 35 mg. and found the results were entirely normal. There was no effect on the prothrombin. We did not do any test for fibrinogen.

SUBACUTE BACTERIAL ENDOCARDITIS CONFINED TO THE RIGHT SIDE OF THE HEART AND ENDARTERITIS OF THE PULMONARY ARTERY

PAUL S. BARKER, M.D.
ANN ARBOR, MICH.

The usual clinical features of subacute bacterial endocarditis confined to the right side of the heart, and of subacute bacterial endarteritis superimposed on patent ductus arteriosus, are well known. It is also well known that they are extremely variable, that many cases deviate from the typical picture, and that diagnosis is often difficult. Nevertheless, the success of surgery in curing patent ductus in which infection has not yet involved the heart valves makes exact diagnosis imperative. For aid in this problem, a study of the University Hospital cases and a review of the literature were undertaken.

The physical signs usually indicate clearly a lesion of the right side of the heart or patent ductus arteriosus. Sometimes the signs are atypical, the murmurs confusing or absent. Murmurs often change and occasionally disappear during the course of the disease, due probably to changes in the vegetations which may occlude a patent ductus or alter the direction of flow through a septal defect by obstructing the pulmonary artery. Systolic and, occasionally, diastolic murmurs may be heard in the mitral or aortic area in the absence of lesions of these valves. Conversely, these valves may be involved without producing characteristic signs. In endarteritis complicating patent ductus, involvement of the aortic and mitral valves is usually obvious, but involvement of the pulmonic valve is often obscured.

Signs of infection are present, such as fever and tachycardia. Anemia usually appears early. The leucocyte count varies but is usually moderately elevated. Abdominal discomfort, anorexia, nausea, wasting, and cough are common. Blood cultures are usually positive; sometimes they are sterile, especially early in the disease.

Infarction of the lungs is usually, but not always, present. Peripheral emboli in the greater circulation are rare and require special explanation. In some instances, the flow through a septal defect or a patent ductus may be altered so that it is from right to left. In other cases with no abnormal communication, one may speculate regarding the possibility of infected pulmonary infarcts causing pulmonary venous thrombosis which in turn gives rise to peripheral emboli. Enlargement of the spleen is common and is usually due to acute splenic tumor accompanying the infection or to passive congestion, occasionally to infarction. Albuminuria and microscopic hematuria are common, and autopsy usually reveals glomerulotubular nephritis; rarely renal infarction or focal embolic nephritis is discovered. Petechiae sometimes appear, occasionally purpura.

DISCUSSION

DR. M. J. SHAPIRO, Minneapolis, Minn.—I would like to comment on the diagnosis of patent ductus without a typical murmur. One can conjecture as to whether he has a patent ductus if the murmur is not typical but I would suggest that such a diagnosis be not made preoperatively. If the murmur is not typical and surgery is done on many of these patients, a lot of mistakes will be made.

A patient with subacute infection superimposed on a patent ductus should be operated because he has nothing to lose. Even with penicillin and the sulfonamides we have nothing available. In a large percentage of cases of subacute bacterial endocarditis, cure has been obtained by surgery alone.

DR. C. J. WATSON, Minneapolis, Minn.—I should like to ask Doctor Barker whether he has encountered right-sided bacterial endocarditis in patients with pulmonary tuberculosis and whether he knows if tubercle bacilli may cause subacute bacterial endocarditis. We recently had a patient who had an extensive pulmonary tuberculosis with cavities. He presented a very loud systolic murmur in the pulmonic area but had no evidence otherwise of subacute bacterial endocarditis with the exception of an enlarged spleen. This patient was discharged to a tuberculosis sanitarium at some distance. We were told subsequently that soon after arriving there he developed a severe purpura and at autopsy he had vegetations on the pulmonary valve, but no bacteriologic studies were made. We did not presume that this was a tuberculous endocarditis but it raised the question in our minds and I would like to know what you think about it.

DR. BARKER (closing).—In one of our cases a clinical diagnosis of subacute bacterial endocarditis in an interventricular septal defect was made. The patient left the hospital against advice and did not continue with sulfonamide treatment. Later, he turned up as a patient with tuberculosis of the lungs which he did not have at the time he had the bacterial endocarditis. He had recovered from the bacterial endocarditis and had normal temperature and negative blood cultures. In the literature there are reported several patients with right-sided subacute bacterial endocarditis and tuberculosis of the lungs and one with tuberculosis of the kidneys. I did not encounter any with tubercle bacilli implanted on the valves or on the endocardium.

I agree that operation should be restricted to patients with typical murmurs. Peripheral embolism is relatively uncommon and should suggest involvement of the mitral or aortic valves. In our limited experience some patients have responded to penicillin therapy and apparently have recovered from their infection following its use.

A CORRELATION OF COMPOSITE LIVER FUNCTION STUDIES WITH HISTOLOGIC CHANGES IN THE LIVER AS NOTED IN BIOPSY MATERIAL

F. W. HOFFBAUER, M.D.
MINNEAPOLIS, MINN.
(INTRODUCED BY C. J. WATSON, M.D.)

Clinical studies of liver function in various forms of jaundice and liver disease indicate the relative futility of using but one or two procedures intended to measure liver function. In the present study a battery of liver function tests has been employed, and the results have been recorded graphically in the form of a liver function "profile" or composite record. The present material consists of forty-eight cases in which composite studies were made and in which a total of fifty-three needle biopsies of the liver were carried out. This includes twelve cases in which, because of relative inaccessibility of the liver, the biopsy was done at the time of laparoscopy. This is to say that a biopsy needle was inserted directly through the anterior abdominal wall; the insertion into the liver was guided by visualization through the peritoneoscope. The employment of a needle allows sampling of liver tissue to a greater depth than is per-

mitted with the peritoneoscopic biopsy forceps, thus eliminating the objection to the latter method that only subcapsular material is obtained. For the ordinary biopsy (thirty-six cases), in the presence of an enlarged and easily palpable liver, the procedure was carried out at the bedside using an 85 mm., 14-gauge Silverman needle, as first employed for this purpose by Tripoli and Fader. The biopsy under peritoneoscopic control was done with a modified 180 mm. Silverman needle of the same gauge.

The composite study of liver function has included the following procedures for the nonjaundiced patient: quantitative fractional serum bilirubin; hippuric acid (one hour, intravenous method); fractional serum proteins; cephalin-cholesterol flocculation, bromsulfalein 2 mg. per kilogram with twenty-minute reading; urine urobilinogen in milligrams per twenty-four hours. In the jaundiced subject the same procedures, with exception of the bromsulfalein test, are used, together with the total serum cholesterol, the serum phosphatase, the Quick prothrombin time (before and after vitamin K), and the feces urobilinogen (milligrams per day). The renal function factor in the hippuric acid test is evaluated by means of a simultaneous one-hour phenolsulfonephthalein excretion test (1 c.c. of the dye is injected together with the sodium benzoate). In instances where the results with these standard procedures were conflicting or doubtful, supplementary and more sensitive tests were carried out. These included the bromsulfalein 5 mg. per kilogram thirty- and forty-five minute reading and the bilirubin tolerance test in the nonjaundiced subjects; the intravenous galactose test, stercobilin tolerance test, and urine coproporphyrin in both jaundiced and nonjaundiced subjects.

It is believed that the conjunction of the composite liver function study with liver biopsy has permitted much additional insight into the pathologic physiology of jaundice and liver disease. From these studies it has become increasingly apparent that cirrhosis of similar anatomic extent may exhibit strikingly different degrees and types of liver functional impairment. The concept of a pericholangitic type of hepatitis with normal hepatocellular function has become increasingly clear as a result of these correlations. The diagnosis of obscure or uncommon liver disorders, as, for example, primary amyloidosis, has been established by biopsy in cases in which the liver function profile, alone, although abnormal, was not diagnostic.

The study indicates that if a large enough series of correlations of the above type become available, the resulting patterns may provide information both as to the anatomic type of hepatic disease, and to the prognosis.

DISCUSSION

Lieutenant Colonel Johnson McGuire, Cincinnati, Ohio.—I would like to ask if there has been any mortality in securing these biopsies.

Dr. C. J. Watson, Minneapolis, Minn.—I might add a word about liver biopsy, having watched Doctor Hoffbauer's procedure repeatedly. He has done somewhat more than sixty-five without untoward incident. An accident did occur in one case where the biopsy was done by a visiting physician, although under supervision. In this instance the needle undoubtedly went through the thin left lobe of the liver and damaged a vessel behind it. An alarming hemorrhage resulted which, however, stopped spontaneously. The patient recovered completely. If one limits the procedure to cases where the liver is easily pal-

pable below the right costal margin, and obviously just beneath the abdominal wall, the biopsy is believed to be quite safe. In other cases needle biopsy can be carried out satisfactorily under peritoneoscopic control. When observed through the peritoneoscope, the amount of bleeding is small, a few drops welling up on the surface after removal of the needle, promptly followed by clotting and cessation of bleeding. The insertion of the needle is relatively painless if the parietal peritoneum is first carefully anaesthetized.

DR. HOFFBAUER (closing).—Needle biopsy of the liver has not been attempted in any patient where there was a suspicion of intra-abdominal or intrahepatic suppuration. The presence of a hemorrhagic tendency has also been regarded as a contraindication. No fatalities have occurred as a result of this procedure.

Biopsy of the liver, as carried out by the surgeon at the time of operation or by the endoscopist with the peritoneoscope forceps, has been criticized on the basis that if the material is secured from just beneath the capsule it may not reflect the actual structure of the liver elsewhere. This can be avoided by the operator if an attempt is made to remove tissue from a sufficient depth into the liver. The Silverman biopsy needle permits penetration to a depth of several centimeters and is therefore advantageous.

THE CEPHALIN-CHOLESTEROL FLOCCULATION TEST

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ST. LOUIS, MO.
(INTRODUCED BY CARL V. MOORE, M.D.)

The present communication is an attempt to evaluate the cephalin flocculation test under a variety of circumstances. From a series of 1,500 tests, 500 were done on the sera of patients whose diagnoses were established beyond any reasonable doubt. The results may be summarized as follows:

	NEG.	POS.
I. Diffuse parenchymatous disease of the liver	2.8	97.2
II. Discrete lesions of the liver	46.7	53.3
III. Suspected lesions or dysfunction of liver	62.8	37.2
IV. No known lesion or dysfunction of liver	81.9	18.1

Negative reactions did occur in the presence of diffuse liver disease but the lesions were invariably minimal. Flocculation was not encountered in normal healthy controls, but in the presence of infection, allergic disease, and during the puerperium or neonatal period positive reactions were encountered which are thought to be "false positives." In twelve of thirteen instances studied, the sera of mother and newborn infant ran a parallel course suggesting that the causative agent passes the placental barrier. These facts are not incompatible with Hanger's hypothesis that the flocculation is due to an abnormal globulin constituent, although no correlation was possible with the albumin-globulin ratios. It is suggested that as in the case of the flocculation tests for syphilis, experience will make apparent those situations in which the test is not reliable.

The test is of greatest value in acute hepatitis, catarrhal jaundice, and cirrhosis or in the presence of any other diffuse parenchymatous liver disease. In these situations the test has prognostic as well as diagnostic value. The flocculation test is of no value in the differentiation of obstructive and nonobstructive jaundice. Other tests, particularly the bromsulfalein test is more useful in detecting focal hepatic disease.

BILIVERDIN ICTERUS

MAJOR E. A. LARSON, MEDICAL CORPS, ARMY OF THE UNITED STATES, AND G. T. EVANS, M.D. (BY INVITATION), MINNEAPOLIS, MINN.

A direct colorimetric method for the determination of serum biliverdin is described; it employs the Evelyn colorimeter and depends for its validity on the increasing light absorption of biliverdin in passing from green to red wave lengths, as compared with the decreasing or unchanging absorption of bilirubin, reduced hemoglobin, and oxyhemoglobin in passing through the same range. Methemoglobin must be specifically excluded.

Normal sera contain no detectable biliverdin (less than 0.05 mg. per cent). Ninety-three observations were made on the sera of sixty-three consecutive patients with jaundice. The biliverdin values ranged from zero to 2.2 mg. per cent.

When biliverdin is present, the general trend is for the higher values to occur in patients with the higher values for total bilirubin; thus, the six highest values for biliverdin (over 1 mg. per cent) were in patients (three carcinomatous obstruction; two, common duct stone; one, catarrhal jaundice) having total bilirubin above 17 mg. per cent (average, 24.4 mg. per cent).

A significant level of biliverdine was found in every patient with the following: (a) carcinomatous obstruction of the common bile duct (twenty-six); (b) catarrhal jaundice (four); (c) cirrhosis with jaundice (five); (d) common bile duct stricture (five). Among the patients mentioned in whom biliverdin was uniformly present there were fourteen with total bilirubin under 10 mg. per cent (average, 5.9 mg. per cent); compared with this it is worthy of note that of the ten patients with common bile duct stone, five with an average total bilirubin of 7.1 mg. per cent had no biliverdin in their sera.

The remaining thirteen patients (six transfusion reactions; three, hemolytic anemia; one, leucemia; one, Hodgkins' disease; one, Banti's syndrome; one, cardiac decompensation) with average total bilirubin of 7.6 mg. per cent (range 3 to 30 mg. per cent) all had zero biliverdin.

The green tint of the patient was noted in those having as little as 0.3 mg. per cent biliverdin (total bilirubin, 9 to 20 mg. per cent).

Serial observations in fourteen patients showed that biliverdin can change markedly in from one to five days without respect to the direction of change of the total jaundice. In three of these patients, during a period when there was a notably high caloric intake, the biliverdin was observed to disappear despite absence of marked change in the total jaundice.

In general it may be said that biliverdinemia is a further evidence of regurgitation jaundice and has not been observed in hemolytic (retention) jaundice. The absence of biliverdinemia speaks against the diagnosis of jaundice due to cancer.

PRIMARY SPLENIC PAN-HEMATOPENIA

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COLUMBUS, OHIO

The physiologic role of the spleen as a reservoir and regulator of the circulating cellular elements in the blood is well established. The selective pathologic sequestration and destruction of red blood cells (congenital hemolytic icterus), platelets (thrombocytopenic purpura), and granulocytes (primary splenic neutropenia), respectively, associated with an hyperplasia of highly phagocytic R-E cells, with or without demonstrable splenic enlargement, may each be treated successfully by splenectomy. Secondary, usually asymtomatic, decreases in one or more of the cell types not primarily attacked by the splenic phagocytes have been noted and reported in our previous communications dealing with each syndrome as a separate entity. If this erratic pathologic physiology of the spleen is the principle underlying etiology in the conditions described, it should not be surprising to encounter certain individuals in whom this organ failed entirely to discriminate between the blood elements passing through its confines, with a resulting peripheral hematologic and clinical picture superficially resembling the pan-marrow hypoplasia in aplastic anemia. It is the purpose of this report to define and differentiate such a newly recognized syndrome, to suggest a diagnostic designation descriptive of the fundamental underlying mechanism, to present clinical examples, and to recommend splenectomy as an effective therapy.

"Primary splenic pan-hematopenia" occurs both as a chronic relapsing, and as a relatively acute, clinical syndrome, either associated with or independent of the congenital or acquired splenic dyscrasias previously described. The adrenalin test will reveal an abnormal excessive sequestration of all normal circulating elements by the spleen through a manyfold temporary increase above the base line in the peripheral circulation. Sternal bone marrow aspiration will show pan-marrow hyperplasia of all normal elements without pathologic maturation arrest or significant qualitative abnormalities, or foreign cell invasion, a finding in sharp contrast with the acellular marrow in hypoplastic anemia, or the hyperplasia in metastatic myelophthisia.

At least five more or less clear-cut examples of this syndrome have been encountered in our series of 170 splenectomies for splenic dyscrasias. The first case is that of a 14-year-old girl, apparently normal at birth, but from 6 months of age until seen in our Clinic in October, 1943, she had suffered more or less constantly from profound nonicteric anemia, leucopenia with recurring superficial infections and nonspecific febrile episodes, and easy bruising. A diagnosis of hypoplastic anemia was made when all hematogenic therapy failed, but no blood transfusions were ever given and complete aplasia obviously never occurred. The first available blood study was made at 29 months of age: W.B.C. 4200, R.B.C. 1,210,000; Hb. 4.5 Gm., PMN 29 per cent, PME 1 per cent, lymphocytes 65 per cent, monocytes 1 per cent. On admission Oct. 13, 1943: W.B.C. 1600, R.B.C. 1,030,000, Hb. 3.5 Gm., reticulocytes 3 per cent, platelets 228,000 (normal 750,000), hematocrit 10, MCV 97 c.mm., MCH 34, MCHC 35 per cent; corrected sedimentation index 0.2 mm. (normal); erythrocyte fragility range .412-.341 (normal); icterus index 7; van den Bergh normal; supravital differ-

ential, PMN 36 per cent, PME 18 per cent, lymphocytes 38 per cent, monocytes 8 per cent. Chest x-ray, electrocardiogram and liver and kidney function tests showed no abnormalities; basal metabolic rate +15. *Adrenalin test:* W.B.C. 1,450 to 10,250; R.B.C. 1,300,000 to 1,700,000; Hb. 5.3 to 6.5 Gm., platelets 313,000 to 911,000. *Sternal bone marrow:* hyperplastic grossly; myeloid: erythroid ratio, 4:1; supravital differential; neutrophilic myelocytes "C" 67 per cent, eosinophilic myelocytes "C" 20 per cent, lymphocytes 5 per cent, elasmatocytes 2 per cent, megakaryocytes 3 per cent; normoblasts 30 per cent, late erythroblasts 20 per cent, early erythroblasts 22 per cent, megaloblasts 28 per cent, no foreign or atypical cell types seen. *Splenectomy*, Oct. 21, 1943, with 1 c.c. adrenalin injected directly into the splenic artery prior to ligation of splenic pedicle: preoperatively, W.B.C. 2100, PMN 798; postoperatively W.B.C. 17,000, PMN 7,140; R.B.C. 1,100,000 to 2,010,000; Hb. 4.3 to 7.8 Gm.; platelets 255,000 to 1,157,000. Spleen weight 475 Gm.; no perisplenitis or adhesions, no infarction; supravital studies, hypersequestration of PMN, PME, R.B.C., and platelets with increased number and activity of phagocytic elasmatocytes; no hematopoiesis. By fourteen days postoperatively: W.B.C. 9,000, PMN 3,780; R.B.C. 3,050,000, Hb. 9.5 Gm.; platelets 4,740,000/c.mm. Clinical improvement was dramatic and has continued to the present time. First normal menses, Jan. 30, 1944.

Mrs. D. K., aged 24 years, was admitted as a medical emergency to the Hematologic Service, University Hospital, with vague symptoms for six months, subacute for four weeks, and acute for seventy-two hours. Increasingly profound prostration with tachycardia, palpitation, dyspnea, precordial pain, marked pallor with questionable fluctuating icterus during the last ten days, and nausea and vomiting for seventy-two hours, were the presenting complaints. The spleen extended to the umbilicus. F. H. negative. Admission hematologic data: W.B.C. 2,100, PMN 420, myelocytes "C" 210; R.B.C. 820,000, Hb. 2.8 Gm., platelets 16,000 reticulocytes 69 per cent, icterus index 100, van den Bergh indirect, 3.69; urobilinogen, strongly positive. *Sternal bone marrow:* grossly hyperplastic wiith microscopic pan-hyperplasia for all normal elements. Splenectomy was successfully accomplished five hours after admission; preoperative and postoperative data, without blood transfusion, were as follows: W.B.C. 2,100 to 4,950; R.B.C. 1,010,000 to 1,990,000, Hb. 2.8 to 5.6 Gm., platelets 16,000 to 114,000. Hematologic and clinical improvement continued in parallel without complications, the patient being discharged on the twelfth postoperative day. A summary chart of the five cases shows similar cellular response following splenectomy.

DISCUSSION

Dr. Howard L. Alt, Chicago, Ill.—These observations of Doctor Doan are of unusual interest. I saw a patient last July, a woman aged 62 years, with a similar condition. The red blood count was 1.56 million; white blood count, 2,900, and platelets, 55,000. The icterus index was 12 units and the reticulocyte count, 4.8 per cent. The bone marrow was normally cellular, the myeloid:erythroid ratio was 4:1, and there was some shift toward maturity in the normoblastic cells.

Following splenectomy early in August there was gradual improvement in the blood picture and all the elements returned to normal levels in a month. Because of the nature of the bone marrow pattern and the slow recovery, it was

thought that the hematopenia was due to splenic inhibition of bone marrow function.

In going over some of our cases of pancytopenia seen in the past, there are several that could be classified as primary splenic panhematopenia. They were originally diagnosed as primary refractory anemia with hypercellular marrows. Splenectomy deserves more extensive trial in this group of patients.

Dr. Frank H. Bethell, Ann Arbor, Mich.—I should like to ask Doctor Doan how he interprets the results of the adrenalin test in determining the indications for splenectomy and if he performs puncture biopsies of the spleen on his patients before advising splenectomy.

Dr. R. W. Heinle, Cleveland, Ohio.—We have had two cases similar to Doctor Doan's which have been relieved by splenectomy so far as the neutrophile count is concerned. In one patient a pre-existing anemia was improved after splenectomy and in one it was not. I wonder how Doctor Doan explains the absence of evidence of hemolysis? It was absent in our patients and in all of Doctor Doan's patients except the last one reported this morning.

If the sole pathologic physiology is sequestration in the spleen, I wonder why there is sometimes considerable delay in the return of the neutrophiles after splenectomy? This was true, particularly, in our two patients; in one the neutrophiles increased slowly over a period of one year. It would seem to me that if sequestration were the only factor, the neutrophiles should increase promptly after splenectomy. Also, in view of the well-known abnormal sphericity of the erythrocytes in familial hemolytic anemia, I wonder whether splenic sequestration can be considered to be the principal disturbance in this disease Similarly, it is not possible to explain all the features of idiopathic thrombocytopenic purpura on this basis.

Dr. C. J. Watson, Minneapolis, Minn.—I think it might be emphasized that hemolysis does occur in excess in an appreciable number of cases of what is designated as refractory or aplastic anemia. If one studies hemoglobin metabolism in patients with this diagnosis it will often be found that there is an increase of red cell destruction. In our experience this has commonly been cyclic in character, sometimes in excess of a two- to fourfold magnitude and then again in a fairly normal range. The reticulocytes are not increased as they are in the characteristic cases of hemolytic jaundice. On the other hand, they are often not decreased as one might expect them to be with bone marrow hypoplasia. Counts of 1 to 3 per cent are not uncommon. We have had a few such patients splenectomized but we have not seen any enduring benefit following splenectomy. We have a record of one patient with a remission of about four months. The blood picture did not return to normal but improved so markedly that transfusions were unnecessary. Then the anemia recurred and the patient went on to exitus. We have evidently not observed the type of case to have had the good results Doctor Doan has noted.

Dr. Doan (closing).—In answer to Doctor Bethell, the adrenalin test, we feel, will usually reveal quite accurately the type and degree of cell sequestration which is currently occurring in the splenic parenchyma. This is approximately proportional to the size of the spleen. Furthermore, at the operating table it has been shown that the total number of circulating cells in the splenic artery is greater than the number found in the splenic veins, confirming the reservoir function of this organ. The histologically verified presence of increased numbers of highly phagocytic clasmatocytes, containing recognizable blood cell elements corresponding to the specific cellular deficiencies which exist in the peripheral blood, give good reason to suspect this mechanism of abnormal cell destruction. When the bone marrow is found to be hyperplastic for the identical cell types, without evidence of delayed or abnormal maturation, an unusual

demand for these elements may be assumed. Finally, when successful splenectomy is followed by an immediate and sustained increase in all circulating blood cell elements, the primary pathologic role of the spleen in such syndromes would seem to be firmly established.

We prefer not to risk intraperitoneal hemorrhage from direct needle aspiration of the splenic parenchyma through the intact abdominal wall unless the adrenalin test and sternal marrow aspiration together fail to reveal the desired information.

With reference to Doctor Heinle's remarks, I did not mean to infer that splenic sequestration and clasmatocytic destructive phagocytosis were the sole mechanisms which may contribute to circulating blood cell deficits. There are, of course, other well-established factors which must always be evaluated. Reticulocytosis, or an increased icterus index with positive indirect van den Bergh, are not necessary as criteria of excessive red cell destruction or hemolysis. Doctor Watson emphasized this in his discussion. The hepatic and renal thresholds for pigment disposition are extremely important in this regard. As has repeatedly been emphasized, the integrity of the bone marrow must be known in each individual case. When the spleen is the sole primary pathogen, complete and permanent recovery follows splenectomy; when secondarily contributory only, partial re-equilibration only may be expected.

THE ANEMIA IN EIGHT ADULT PATIENTS WITH SCURVY

Richard W. Vilter, M.D., and Robert M. Woolford, M.D. (By Invitation)
Cincinnati, Ohio

In the Cincinnati General Hospital recently we have observed ten male adults with scurvy, eight of whom had anemia. Hemolysis appeared to be one causative factor because of the accompanying reticulocytosis and moderate hemolytic jaundice. There was no evidence of iron deficiency or of external bleeding. In each case the anemia and the other lesions of scurvy responded to the administration of vitamin C. These observations are reported because the existence of a specific anemia of scurvy has been questioned.

Red blood cell counts ranged from 1.74 to 3 million and hemoglobin levels from 5.8 to 10.5 Gm. The cells were normocytic normochromic or moderately macrocytic hyperchromic. The white count was under 6,000 in five patients. Differential white counts were normal. Platelets occurred in normal numbers. Initial reticulocyte levels ranged from 3 to 10 per cent except in two patients in whom initial counts of 1 per cent were obtained.

Bone marrow obtained by sternal aspiration appeared hypocellular in some patients, normally cellular or slightly hypercellular in others. There was a relative increase in the number of red cell progenitors, the majority of which were at the normoblast and late erythroblast maturation levels. The granulocyte series was cytologically normal.

Icteric indices ranged from 10 to 22; the urine contained excess urobilinogen but no bile; and the blood serum gave the indirect van den Bergh reaction in each instance. The extent of ecchymoses, however, could not be correlated with the degree of jaundice, nor with the severity of anemia, and no increase in ecchymoses occurred after the patients were hospitalized.

In the hospital these patients were fed a diet free of vitamin C and low in the vitamin B complex. Specific medication was withheld for from two to

fourteen days until mental torpor and impending shock made the administration of vitamin C imperative.

Before vitamin C administration was begun, erythrocyte counts and hemoglobin levels remained unchanged in six patients and decreased in two although the reticulocyte counts remained elevated or rose slowly.

After daily administration of 500 mg. of vitamin C, two patients showed a rise in reticulocytes from 5 to 10 per cent and from 2 to 19 per cent, the peak value, on the sixth and tenth days, respectively. In the other six patients, initial high reticulocyte levels were maintained for six to ten days. Red blood cell and hemoglobin increases began one week after the initial dose of vitamin C. Within three weeks, the levels reached 3.5 million and 12.5 Gm. or more in all patients. Icteric indices became normal when the red cells began to rise. Repeated bone marrow aspirations on four patients showed reversion to normal.

DISCUSSION

Dr. C. J. Watson, Minneapolis, Minn.—These are extremely interesting and instructive cases but I must object to the view that they are necessarily hemolytic. I agree fully that the findings suggest hemolytic anemia. Urobilinogenuria, however, does not suggest hemolysis. There are many clear-cut cases of hemolytic anemia without urobilinogenuria, and, conversely, there are many with urobilinogenuria without increased hemolysis. The idea that urobilinogenuria is pathognomonic of hemolytic anemia should be abandoned. The findings suggest that there was considerable hepatocellular dysfunction. In addition to that, there may have been hemolysis. I agree that the increased reticulocytes are suggestive of the latter.

Dr. John L. Stifel, Toledo, Ohio.—Could salicylates have been an etiologic factor in any of these patients? I have in mind a patient who twice developed anemia similar to this after having taken a course of salicylates for rheumatoid arthritis. The second time she developed this in spite of large doses of ascorbic acid.

Dr. Armand J. Quick, Milwaukee, Wis.—I would like to ask whether the bleeding time test was done, also a tourniquet test, and, if positive, how soon the latter responded after vitamin C therapy.

Question: I would like to ask if a fragility test was done.

Dr. Stanley E. Dorst, Cincinnati, Ohio.—Doctor Vilter, earlier in his discussions, indicated that previous investigators studying C deficiencies alone did not encounter the symptoms he describes. I wonder whether or not he was able to determine any reasons for the discrepancies between their results and his conclusions.

Dr. Vilter (closing).—I expected to have Doctor Watson question the data favoring hemolysis. Since June we have been waiting for another patient with scurvy so that we might obtain stool urobilinogen levels. Unfortunately such a patient has not appeared.

Liver function tests were done because we wondered whether hepatic dysfunction was responsible for the urobilinogenuria. The prothrombin times, bromsulfalein excretion, and cephalin flocculation tests were essentially normal. The moderately low serum albumin levels could easily be explained by dietary protein deficiency.

There was no specific history of salicylate ingestion, and I do not believe salicylism played any part in the purpura or anemia.

We did not carry out bleeding and clotting times routinely. Those that were done were normal. Tourniquet tests were abnormal, but we did not repeat them during treatment. The tourniquet test is not sufficiently specific for use in the study of scorbutic patients.

I have wondered many times why our results and those of Mettier, Minot, and Townsend differ from Loesner's and others who believe that vitamin C plays no part in hematopoiesis. It is possible that those patients who had hematologic remissions before vitamin C was administered differed from our patients in the degree of vitamin C depletion. All our patients who were put to bed on a diet very low in vitamin C invariably became much worse. Loesner apparently had no such difficulty with much longer control periods than ours. Possibly bed rest alone led to improvement. Similar remissions have been observed frequently in patients with pellagra when put to bed on a B complex-deficient diet.

THE INFLUENCE OF DICUMAROL ON PLATELET ADHESIVENESS

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MADISON, WIS.

In 1941 H. P. Wright presented a method for measuring the adhesiveness of platelets, that is, their tendency to adhere to each other or to surfaces with which they come in contact. She reported that a number of in vitro anticoagulants would cause a decreased adhesiveness and that the decrease was proportional to the amount of anticoagulant used.

The method consisted of rotating blood in glass tubes and making platelet counts at various intervals of time. The tube was constructed in such a way that the sides, which were formed by cover slips held in place with petroleum jelly, could be removed and stained. The rate of fall of the platelet count was taken as a measure of adhesiveness; the less rapid the fall, the less sticky were the platelets.

In this study an attempt was made to determine whether dicumarol, an in vivo anticoagulant having no in vitro action, would cause a similar decreased adhesiveness.

Dicumarol was given to each of thirty-three patients for a period of six days. The dosage was gauged by means of prothrombin time determinations in which Pohle and Stewart's modification of the Quick method was used. Usually, four determinations of platelet adhesiveness were made on each patient, one before the administration of the drug, two during, and one afterward, when the effect was beginning to decline.

It was found that dicumarol produced a definite decreased adhesiveness as measured by the method of Wright. In general, the greatest decrease in platelet adhesiveness corresponded to the greatest prolongation of prothrombin time. The drug produced no change in platelet count per se, an observation previously reported. The data presented constitute additional evidence in favor of dicumarol as a prophylactic agent of thrombosis.

DISCUSSION

DR. RICHARD K. RICHARDS, Chicago, Ill.—I would like to ask Doctor Meyer if he believes there is any relation between platelet adhesiveness and blood viscosity and, second, how heparin affects the adhesiveness with the same circumstances and the same technique.

DR. MEYER (closing).—Helen Wright noted that heparin decreased the adhesiveness of the platelets; proportional to the dose of heparin, it decreased the adhesiveness but did not lead to complete disappearance of this function.

I cannot answer the question regarding blood viscosity.

BLOOD AND BONE MARROW FINDINGS IN INFECTIOUS MONONUCLEOSIS

LOUIS R. LIMARZI, M.D., CHICAGO, ILL., LIEUTENANT JEROME T. PAUL, LIEUTENANT COLONEL ROBERT M. JONES, MEDICAL CORPS, ARMY OF THE UNITED STATES (BY INVITATION), AND H. G. PONCHER, M.D. (BY INVITATION), CHICAGO, ILL.

Detailed studies of the blood and bone marrow were performed on a group of twenty-five patients with infectious mononucleosis during various stages of the disease. Eighteen men and seven women comprised the group. Fourteen ranged in age from 16 years to 26 years; four were less than 16 years of age, and one was over 30 years of age. No Negroes were observed in this series.

The leucocyte count varied between 4,000 to 22,000; in ten patients the count was less than 10,000 and two of these had a white count below 5,000. Only one patient had a count of over 20,000. A normocytic anemia was seen in three patients and a severe microcytic and hypochromic anemia was observed in another. The platelets were normal in number in most patients and moderately increased in several. The sedimentation rate was increased in sixteen patients. The atypical or abnormal lymphocytes which characterize the disease varied between 30 and 82 per cent. Moderate to marked toxic neutrophiles with a moderate shift to the left in the neutrophilic picture was seen in all patients.

The heterophile antibody reaction was positive in thirteen patients, and negative in six; the test was not performed in the remaining six patients. A false-positive Wassermann reaction was not observed.

The bone marrow was hyperplastic in twenty patients and a quantitatively normal marrow was observed in five. The erythroid tissue was normal, except in the patient with the microcytic and hypochromic anemia in whom the bone marrow revealed a normoblastic hyperplasia. The megakaryocytes were normal or increased in number and showed a normal maturation dispersion. The myeloid elements besides being increased in number revealed a moderate to marked degree of granulopoietic immaturity. Immaturity was never carried to the stage of myeloblastic involvement. The atypical lymphocytes of the peripheral blood were conspicuously absent from the concentrated bone marrow material. The presence of a few atypical lymphocytes as well as a few normal lymphocytes, monocytes, and most of the mature granulocytes was due to sinusoidal dilution (peripheral blood). There is no evidence that the atypical lymphocytes which characterize infectious mononucleosis have their origin from cells in the bone marrow. The atypical lymphocytes lack the malignant characteristic that is seen in cases of leucemia, where the lymphocytes replace and crowd out the normal bone marrow elements.

One case of infectious mononucleosis (heterophile titer of 1:1792) was seen in a 25-year-old female with pulmonary tuberculosis. The leucocyte count

reached a peak of 15,500 with 69 per cent atypical lymphocytes. The bone marrow was in the hyperplastic group. The infectious mononucleosis cleared up, but the pulmonary tuberculosis became progressively worse and eventually required surgical therapy (thoracoplasty).

It is concluded that (1) in spite of the large number of atypical lymphocytes in the peripheral blood in infectious mononucleosis, the bone marrow is not involved; (2) the bone marrow reveals a myeloid hyperplasia and immaturity. Either the "toxic agent" acts on the bone marrow to produce a myeloid hyperplasia and suppresses their delivery to the peripheral blood or the suppression of the myeloid cells results in a myeloid hyperplasia; (3) the atypical lymphocytes in infectious mononucleosis show none of the metastatic or replacement characteristics of leucemic cells; (4) in contrast, in patients with acute, subacute, and chronic lymphatic leucemia with many abnormal lymphocytes in the peripheral blood the bone marrow reveals moderate to marked replacement of the normal bone marrow elements. The replacement characteristic of leucemic cells in lymphatic leucemia is seen in the bone marrow when the leucocyte count in the peripheral blood is leucopenic, normal, or increased in type; and (5) sternal puncture is of diagnostic aid in differentiating benign and malignant types of lymphocytosis.

INFLUENZAL ENCEPHALITIS

G. O. Broun, M.D., R. O. Muether, M.D., Henry Pinkerton, M.D. (By Invitation), and Margaret LeGier, B.S. (By Invitation)
St. Louis, Mo.

Leichtenstern, in 1890, described an encephalitis believed due to influenza. Since discovery of influenza virus, no reports have appeared of its isolation from clinical cases of encephalitis. The case here reported was apparently caused by a virus resembling in some way influenza virus.

The patient, a white man, 51 years of age, became ill in January, 1944, when influenza was prevalent in St. Louis. His illness was characterized by fever, severe headache, somnolence, anorexia, nausea, and vomiting. There were few neurologic findings, practically no neck rigidity, and conjunctivitis and reddening of the pharynx were the chief physical findings. The patient had a respiratory illness, resembling mild influenza, six weeks prior to onset of headache.

Spinal fluid showed a cell count of 366 leucocytes and 100 erythrocytes per cubic millimeter. Leucocytes were practically all lymphocytes; protein, 160 mg. per 100 c.c.; Kahn and Wassermann tests, negative. Spinal fluid was bacteriologically sterile.

The patient's serum was tested for the titer of antibodies against influenza A and B virus employing the chicken cell agglutination test of Hirst. A titer of 1/1025 against influenza virus type A was found sixteen days after onset of illness, dropping to much lower levels two months later. The titer against influenza type B was much lower.

Spinal fluid was inoculated in eggs previously incubated for twelve days and subjected then to an additional forty-eight hours of incubation. The fluid,

yolk, and embryo of these eggs tested by the method of Hirst showed positive agglutination of chicken cells, a phenomenon suggesting the presence of influenza virus. The positive test persisted through seven series of egg transfers.

The material from the inoculated eggs, after the third transfer, produced pneumonia by intranasal inoculation in mice, the pathologic picture being similar to pneumonia produced by known influenza virus. Many of these pneumonic lungs were bacteriologically sterile. Intracerebral inoculation of this egg material produced a fatal illness in mice with pathologic evidence of a mild encephalitis.

The agglutination of chicken cells by this agent was not inhibited by known influenza type A or type B antiserum but was inhibited by the patient's own serum in a dilution of 1/250. This suggests a serologic difference between this agent and known strains of influenza virus. It resembles influenza virus in its ability to agglutinate chicken cells and in the pathologic lesions produced.

DISCUSSION

Dr. Thomas Francis, Jr., Ann Arbor, Mich.—I think it quite important to emphasize the reservation that has been made in the presentation of this evidence. Regardless of how many similar agents one can show, one cannot say it is the influenza virus. There are many things that might be equally attributed to mouse pneumonia virus. It has been reported by one group that mouse pneumonia virus will cause agglutination of mouse red cells.

In our experience with a large group of spinal fluids from individuals with mild neurologic disturbances at the time of illness, we were unable to get any evidence of an encephalitic virus being present. I think it is still important to accept this as encephalitis due to an undesignated virus until the evidence for influenza virus is more precise. From the abstract I think it is a question whether this might be called encephalitis clinically, since the signs are much more suggestive of meningitis than of true encephalitis.

Dr. Broun (closing).—We realize the difficulty which Doctor Francis suggests, namely, that there are viruses which occur spontaneously in mice which will cause pneumonia. We have gone back to the original egg material several times and succeeded in transmitting the disease to mice each time.

We are leaning very heavily on the Hirst chicken cell agglutination test as evidence for the presence of influenza virus. This test was positive in inoculated eggs prior to any mouse passage. Failure of known influenza A and B antiserum to inhibit chicken cell agglutination by this agent means that we cannot demonstrate its serologic identity with either of these strains of influenza virus. It might, however, be a serologically different strain.

I do not think that the clinical picture suggests meningitis more than encephalitis. Usually in meningitis there is a distinct neck rigidity and a higher spinal fluid cell count would be expected. The pathologic picture produced by this agent in the brains of inoculated mice was not that of a meningitis. Known influenza virus gave a pathologic picture both in brain and lungs of inoculated mice similar to that produced by this agent. I realize that these pathologic pictures are not too specific.

EPIDEMIOLOGIC OBSERVATIONS ON THE USE OF GLYCOL VAPORS FOR AIR STERILIZATION*

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The clearly established bactericidal and virucidal activity of glycol vapors on air-suspended microorganisms suggested the use of these materials as a new means for attack on the problem of control of air-borne infection. Studies on the engineering aspects of practical application were carried out, following which we were able to proceed with installation of apparatus for vapor generation and distribution at a military cantonment. This field trial was conducted to determine if the incidence of air-borne disease could be reduced by the treatment of living quarters with triethylene glycol vapor in effective concentration. Studies were made on three groups of 640 men, observed for six-week intervals and equally divided into test and control, the former sleeping in glycol treated quarters, the latter in untreated dormitories. We were able to demonstrate the following findings: (1) the apparatus maintained adequate glycol concentrations (0.0025-0.004 mg. per liter of air) and optimum relative humidity; (2) an over-all reduction in bacterial air contamination was obtained with practically complete abolition of hemolytic streptococci from the treated dormitories; (3) the incidence of positive throat cultures (hemolytic streptococci) fell sharply in the test groups at the end of the six-week observation period; there was evidence of prevention of spread of these organisms in the test quarters; (4) reduction in incidence of those diseases believed to be disseminated by the aerial route was effected; these infections included common cold, catarrhal fever, influenza, measles, mumps, scarlet fever, rheumatic fever, acute tonsillitis, chicken pox, acute sinusitis, and pneumonia. The over-all reduction for the full six-week period was 12 per cent. However, when the final seventeen days of each period was studied a reduction of 64 per cent was noted. We believe this to be due to two factors; one, the possible increase in length of incubation of respiratory infections brought about by the unusual conditions existing in military groups, and two, the lowering of infecting doses (lessening of air contamination) in the treated barracks as compared to the control, thus permitting those individuals with a moderate degree of immunity to escape disease.

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